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Biological Markers for Characterization of Potential Sources of Soil-Derived and Geologic Fugitive Dust

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

AIR RESOURCES BOARD
Research Division

BIOLOGICAL MARKERS FOR CHARACTERIZATION OF POTENTIAL SOURCES OF SOIL-DERIVED AND GEOLOGIC FUGITIVE DUST

Final Report
Contract No. 94-321

Prepared for:

California Air Resources Board
Research Division
2020 L Street
Sacramento, CA 95814

Prepared by:

Kate M. Scow	Principal Investigator
Mary Ann Bruns	Post Graduate Researcher
Kenneth J. Graham	Post Graduate Researcher

Department of Land, Air and Water Resources
One Shields Avenue
University of California, Davis
Davis, CA 95616

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ABSTRACT

Principal sources of soil-derived fugitive dust need to be identified to reduce airborne PM_{10} in California's Central Valley. As a means to differentiate soils and possibly identify sources of fugitive dust, we have developed methods in our laboratory to produce fingerprints from microorganisms in soil. Fingerprinting methods described in this report are based on two classes of biochemical material, fatty acids and nucleic acids, which we extract from soil or dust prior to chemical analysis. Fatty acid analysis can be based either on phospholipid fatty acids (PLFAs), found only in cell membranes of living organisms, or on soil fatty acid methyl esters (SFAME) obtained from whole cells and nonliving biological material. Similarly, nucleic acid analysis can be based either on DNA sequences from individual groups of organisms or on DNA from the entire microbial community. PLFA or SFAME fingerprints consist of percentages of different fatty acids detected as peaks in gas chromatograms, while DNA fingerprints consist of band patterns in laboratory gels used to separate DNA fragments. Both types of fingerprinting methods generate multivariate data (fatty acid percentages or DNA band identities), which can be used in principal component analysis (PCA) to assess similarities among samples. Appendices in this report contain protocols for the extraction and analysis methods we developed.

Approximately 300 soil samples from California's Central Valley, representing numerous land use categories, soil types, crops, and other variables important in determining sources of air-borne dust, were analyzed for their PLFA fingerprints. PCA plots of PLFA fingerprints under different vegetation and agricultural management showed a clear separation between microbial communities in poorly drained and well-drained soils. Redundancy analysis revealed that both soil texture and crop type were significantly correlated with variation in PLFA fingerprints across soils. The relative importance of environmental variables in governing the composition of microbial communities could be ranked in the order: soil type > time > specific farming operation (e.g., cover crop incorporation or sidedressing with mineral fertilizer) > management system > spatial variation in the field. Similar conclusions could be drawn from these PLFA data when they were analyzed with artificial intelligence (AI) programs in a research collaboration with a chemometrics laboratory.

SFAME fingerprints, advantageous because they utilize smaller sample sizes and require one third of the time needed for PLFAs, were performed on a smaller subset of soil samples (approximately 20), because we expected their chromatograms to be less reliable due to overlapping peaks that were difficult to identify. SFAME differentiated the 20 soils similarly but not identically to PLFA. We also evaluated the similarity between PLFA and SFAME fingerprints of source soils and bulk dusts collected from the surfaces of field equipment in two locations. In both cases, PLFA fingerprints of bulk dust and source soils were similar. SFAME fingerprints from bulk dusts and their source soils were more dissimilar to each other than were PLFA fingerprints, although SFAME fingerprints grouped together on PCA plots. Even though these findings cannot be directly extrapolated to establish similarities between airborne PM_{10} and source soils, this

study provided the first step in evaluating fingerprints obtained from dust and a single-source soil. More sophisticated multivariate analysis methods, such as AI programs, will be needed to interpret fingerprints of dust derived from multiple sources.

Our research on nucleic acid-based methods focused on identifying and improving methods to extract DNA from soil and on testing several approaches for analyzing the extracted DNA. It was possible to extract high quality DNA from soils representing a wide range of properties. The RAPD (Randomly Amplified Polymeric DNA) method for analyzing DNA, adapted from methods developed for fingerprinting individual species, proved to be inadequate for fingerprinting the extraordinarily diverse microbial communities in soil. Although TGGE (thermal denaturing gel electrophoresis) showed promise as a means to generate DNA fingerprints from specific groups of soil microbes, more research is needed to optimize this method for fingerprinting whole-community DNA. Further development of DNA-based methods is needed to provide taxonomic explanations for differences in fatty acid fingerprints and to supplement fatty acid fingerprinting in cases where more specific methods are required.

This report indicates that PLFA and SFAME fingerprinting of soil microbial communities will differentiate soils in a reproducible manner, although DNA fingerprinting requires additional development. We found that fingerprints of bulk dusts and their source soils were sufficiently similar to warrant adaptation of fingerprinting technology to PM_{10} studies of fugitive dust. We describe in the final section of this report how fingerprinting methods could be applied to PM_{10} samples collected on filters in the field. We also describe how AI programs could enhance statistical analysis of fingerprint data for source apportionment studies of PM_{10} in the field.

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GLOSSARY OF TERMS AND ACRONYMS

AI	Artificial intelligence
ART-2a	Adaptive resonance theory (neural network approach)
CNL	Crocker Nuclear Laboratory at UC Davis
DNA	Deoxyribonucleic acid
FID	Flame ionization detector (standard detector for gas chromatograph)
GC	Gas chromatograph
GC-MS	Gas chromatograph coupled to mass spectroscopy detector
HPLC	High performance liquid chromatography
IMPROVE	Interagency Monitoring for the Protection of Visual Environments
Kohonen	Kohonen analysis (neural network approach)
MIDI	Microbial Identification, Inc.
NN	Neural network
PCA	Principal component analysis
PCR	Polymerase chain reaction
PLFA	Phospholipid fatty acid (fatty acids found in living cell membranes)
RAPD	Randomly amplified polymorphic DNA (random fingerprinting)
RDA	Redundancy ordination analysis
RISA	Ribosomal intergenic spacer analysis
RNA	Ribonucleic acid
SAFS	Sustainable Agriculture Farming Systems (UCD agronomic research site)
SFAME	Soil fatty acid methyl ester (from dead or living cells or plant material)
SIMCA	Soft independent modelling of class analogy
TGGE	Thermal gradient gel electrophoresis

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1.0 INTRODUCTION

1.1 Background

Soil-derived fugitive dust often constitutes the largest fraction of PM_{10} reaching high levels[1] in California's Central Valley in late summer and early fall. Soil-derived fugitive dust may be generated by agricultural field operations, construction activities, wind erosion, and traffic on unpaved roads. However, the principal source(s) of fugitive dust in the Central Valley have not been determined. Methods have been developed in our laboratory to produce fingerprints from the microorganisms present in soils as means to differentiate soils and possibly identify fugitive dust sources. Biological fingerprinting is based on the fact that soils are a habitat for complex communities of bacteria, fungi, protozoa, and other microorganisms, all of which contain biochemical material that can be extracted and analyzed. The types and relative abundances of extracted biochemicals represent a rich store of multivariate information that can be interpreted as a fingerprint of the microbial community. We have developed fingerprinting methods based on two classes of biochemicals, fatty acids and nucleic acids. Fatty acid analysis can be restricted to phospholipid fatty acids (PLFAs), found only in cell membranes of living organisms, or it can encompass fatty acids from whole cells and nonliving biological material in the case of SFAME (soil fatty acid methyl ester) analysis. Similarly, nucleic acid analysis can be restricted to the retrieval of DNA sequences from very narrow subgroups of microorganisms or expanded to use genetic material from the entire microbial community.

1.2 Objectives

Objectives of this research were to: (1) develop PLFA, SFAME, and DNA fingerprinting methods for microbial communities from soils and dusts; (2) evaluate the reproducibility and discriminatory capabilities of fingerprints obtained from diverse agricultural soils in the Central Valley; (3) compare fingerprints from dusts and source soils; and (4) use multivariate statistical analysis to quantify similarities among fingerprints and to identify factors that exert significant influences on fingerprints.

2.0 MATERIALS AND METHODS

2.1 Collection of samples.

Soil samples were collected from agronomic research plots (top 15 cm) on the UCD campus and in outlying areas. Additional soil samples were obtained from the UCD Air Quality Group's PM_{10} Project[2], which had a collection of air-dried soils from privately owned fields

[1] PM10 is particulate matter with an aerodynamic diameter of 10 microns or less, small enough to enter the human respiratory tract.

[2] Air Quality Group, Crocker Nuclear Laboratory, University of California, Davis. 1994 and ongoing. Sources and sinks of PM10 in California's San Joaquin Valley: A study for the U. S. Department of Agriculture, USDA Contract No. 94-38825-0383.

under different crops in the San Joaquin Valley. We also included soils from other farms and sites in the Central Valley, so that additional crops, soil types, and geographic locations were represented.

2.1.1 SAFS soils.

A study of seasonal, crop, and management influences on fingerprints of the same soil was conducted at the Sustainable Agriculture Farming Systems (SAFS) Project, initiated at the UC Davis Agronomy Fields in 1988. The soils are Reiff (coarse-loamy, mixed, non acid, thermic, Mollic Xerofluvent) and Yolo loams (fine-silty mixed, non acid, thermic, Typic Xerothent).

The 56-plot experiment has a randomized complete block design, with crop rotations as split plots within each main plot for each farming system, with four replications. The farming systems include organic, low input, conventional four-year, and conventional two-year rotations. The organic system relies on organic sources of nutrients obtained from a vetch winter cover crop, manure, seaweed and fish powder. No pesticides are used and the plots are managed according to California Certified Organic Farmers requirements. The low-input system, which is intermediate between the organic and conventional systems, relies on vetch cover crops as a partial source of nitrogen but is supplemented with mineral fertilizers and limited amounts of pesticides. The conventional systems use only mineral fertilizers, some pesticides, and the only organic matter inputs are in the form of stubble and roots from the previous cash crop of wheat and beans. In the study of seasonal and management effects, soils were sampled from tomato plots within each farming system. Samples were collected while cover crops were growing in the organic and low input plots (April 4), after cover crop incorporation in low input and organic systems and manure application to the organic plots (April 18), one week prior to (May 9) and one week after (May 23) mineral fertilizer sidedressing in low input and conventional systems, and two times later in the growing season (July 3 and July 28). In the study of crop effect on soil fingerprint, soils were collected in July, 1997, in all plots. In another study of seasonal changes, samples were collected in tomato plots throughout the year in 1995.

2.1.2 PM_{10} soils.

A collection of soils sampled in the fall of 1994 for the USDA PM_{10} project was made available to us by Dr. Randy Southard. These had been sampled during harvest operations (top 10-15 cm) from soils cropped with cotton, almond, figs, or walnut. The soils had been air-dried, sieved with a 2-mm sieve, and stored in cardboard cartons at ambient temperature. Particle size analysis data and information about the sample sites (e.g., crop, geographic location) were used to select samples for analysis. Sampling information and PLFA sample identification numbers are listed for all soils in Appendix 9-1.

2.1.3 Bulk dust from equipment surfaces and source soils.

Bulk dust fall (total particulates) and source soils were collected at two locations. The first location was a fallow field at the UC Davis Campbell Tract that was being land planed. Dust was collected over a period of one hour in pans taped to a horizontal bar located immediately above the plane (1 m height). Surface soils were sampled volumetrically to a depth of 15 cm from an area represented by one soil map unit (Reiff loam). At the Stone Land site near

Coalinga, bulk dust was collected on June 20, 1997, from the rear horizontal surfaces of a disker that had gone over the east half of Stone Section 02, the wheat crop from which had been harvested two days prior. Surface soils were sampled to a depth of 15 cm from two areas of the section which represented different soil map units, Westhaven loam and Kimberlina fine sandy loam. Bulk dust and source soils were air-dried and stored in cardboard cartons at ambient temperature.

2.1.4 Other soils included for comparison.

Additional agricultural soils from ongoing projects in collaboration with other labs were included in our analyses. These soils were sampled to depths of 10-15 cm and kept frozen until analysis. Soil sources included Capay clay from rice fields near Maxwell (Bossio and Scow, 1997a,b), Biologically Integrated Farming Systems (BIFS) plots near Fresno, Long Term Research on Agricultural Systems (LTRAS) plots in UC Davis Agronomy fields, rotational management fields in Ventura County from Ben Faber, Tinker soils from pine forest area in Sacramento County, and fallow Rindge soils from the Delta region. In addition, samples of sediment from Clear Lake and of household compost were included for comparison

2.2 PLFA extraction and analysis.

Lipids were extracted from soil samples using a mixture of chloroform, methanol, and a phosphate buffer (White et al. 1979). Extracted lipids were reconstituted and separated into three lipid classes (neutral, glyco- and phospholipids) using silicic acid columns. The phospholipids were retained, dried with nitrogen, then trans-esterified to form fatty acid methyl esters. We used the automated Microbial Identification Diagnostic System (MIDI, Inc., Newark, NJ), which consists of a Hewlett Packard gas chromatograph and software for the identification of fatty acids. A gas chromatograph - mass spectrometer in another UCD laboratory was used for confirmation of fatty acid identity. The final protocol is described in Appendix 9-2.

2.3 SFAME analysis.

SFAME (Soil Fatty Acid Methyl Ester) was developed as an alternative method to PLFA analysis in order to get fingerprints from a smaller amount of soil. The final SFAME protocol is described in Appendix 9-3. This protocol takes approximately 1 day to prepare 16 samples in comparison to the 3 days required for PLFA analysis. As little as 300 milligrams of soil can be analyzed by SFAME, whereas approximately 5-8 grams is needed for PLFA.

2.4 DNA-based methods.

2.4.1 Extraction and purification of DNA from soils.

DNA from soil microorganisms was obtained by subjecting soil samples to chemical and physical treatments which lyse microbial cells and allow microbial DNA to go into solution. These direct lysis procedures, which were adapted from Malik et al. (1994) and Zhou et al. (1996), involved enzyme-treatment/freeze-thaw and high-salt/heat-treatment, respectively. Subsequent steps to purify DNA from cellular debris and soil humic acids were chloroform extraction, precipitation of DNA with ethanol, and agarose gel electrophoresis of crude DNA extracts. Portions of gel containing DNA were excised, and residual agarose was removed with either a Prep-a-Gene kit (BioRad Corp., Hercules, CA) or digestion by Gelase enzyme (Epicentre

Technologies, Madison, WI) followed by centrifugal filtration (Micron Separations, Inc., Westborough, MA). DNA was quantified by measuring its absorbance at 260 nm in a Lambda 10 UV/Vis spectrophotometer (Perkin-Elmer Applied Biosystems, Foster City, CA). DNA purity was checked by measuring absorbances at 230 and 280 nm, which indicate contamination with humic acids and proteins, respectively. The resultant DNA was generally of sufficient purity for polymerase chain reaction (PCR) amplification and restriction enzyme digestion. The protocols used to extract and purify DNA for TGGE analysis were adapted from Zhou et al. (1996) and are described in Appendix 9-4.

2.4.2 PCR amplification of DNA.

Two approaches were used to produce fingerprints from microbial community DNA using PCR. The first approach employed RAPDs, or Random Amplified Polymorphic DNA mixtures using the procedure of Malik et al. (1994). Purified DNA extracts were subjected to PCR amplification with random oligonucleotide primers in a GeneAmp 2400 thermal cycler (Perkin-Elmer Applied Biosystems, Foster City, CA). The mixtures of RAPD products from each DNA extract were applied to lanes in agarose gels and separated by electrophoresis (Sambrook et al., 1989). Gels were stained with ethidium bromide so that DNA band patterns in each lane could be visualized as a fingerprint, consisting of the number, location and intensity of RAPD fragments in the gel. Each RAPD fingerprint was based on the different sizes of DNA fragments produced during PCR amplification of community DNA.

In the second approach, we amplified purified DNA with nonrandom oligonucleotide primers complementary to 16S ribosomal RNA (rRNA) genes in bacteria (Pace et al., 1981). PCR amplification was carried out in 25-microliter reaction volumes containing the following: 5 nanograms of purified community DNA in 1X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 9, 1% Triton X-100); 2.5 mM MgCl2; 1.25 mM deoxyribonucleotides, 10 picomole forward primer, 10 picomole reverse primer (Table 2-1); and 1.5 Units of Taq DNA polymerase (Promega Corp., Madison, WI). The GeneAmp 2400 PCR program consisted of 30 cycles of denaturing at 94C for 1 min; primer annealing at 55C for 1 min; and DNA extension for 72C for 1 minute. With this approach, rRNA genes in the bacterial community DNA were amplified to yield a mixture of PCR products reflecting the taxonomic composition of the community.

2.4.3 TGGE fingerprinting of DNA.

A thermal gradient gel electrophoresis (TGGE) system (Heuer and Smalla, 1997) was constructed in our laboratory from a vertical electrophoresis rig fitted with an aluminum block, against which glass plates (42 cm high x 30 cm wide) containing polyacrylamide gel could be clamped. David Paige, in the Department of Land, Air, and Water Resources, designed the thermal block and temperature controlling mechanisms for the system. The upper portion of the aluminum block contained an internal channel (8 mm diameter) through which water was pumped using a Neslab RTE-111 circulating water bath (Neslab Corp., Portsmouth, NH). The bottom portion of the block containing an electrical heating strip connected to a temperature controller (Model 1500, Dwyer Instruments Incorporated, Michigan City, IN). The temperatures of the water bath (for the cooled upper block) and the controller unit (for the hotter lower block) could be adjusted to provide a linear temperature gradient across the length of the gel. We evaluated several different denaturant and polyacrylamide concentrations, running buffer

concentrations, and temperature gradients in attempts to optimize band separation. We also ran PCR reactions using a primer set specific for ammonia-oxidizing bacteria. Gels were stained either with ethidium bromide or with more sensitive silver reagents (Mitchell et al., 1994) to visualize the bands. Gel images were recorded using a charge-coupled-device camera with the BP-M1/722 TWAIN digital imaging kit (Bioimage, Ann Arbor, MI) and evaluated with Photofinish and GPTools image analysis software.

We tested two sets of TGGE primers in PCR reactions with DNA samples extracted from PM10 soils and from bulk dust. Both primer sets successfully amplified DNA from the extracts. One primer set was used to produce fingerprints from the DNA of the whole bacterial community, while the other set was used to produce fingerprints from DNA of nitrifying bacteria (Table 2-1). Prior to running the PCR products on polyacrylamide gels, we calibrated the temperature gradients for separating PCR products from community DNA using all-bacteria primer sets and nitrifying-bacteria primer sets.

Table 2-1. DNA sequences of primers used in PCR amplification of taxonomic genes for soil fingerprinting.

Target group of microorganisms	Forward primer sequence* (5' to 3')	Reverse primer sequence (5' to 3')	References
All bacteria	GC clamp-CCT ACG GGA GCA GCA G (corresponds to E. coli positions 341-357)	CCC CGT CAA TTC CTT TGA GTT T (corresponds to E. coli positions 907-928)	Teske et al. (1996)
Nitrifying bacteria	GC clamp-AG$^{A}/_{G}$ AAA GC$^{T}/_{A}$ GGG GAT CG (corresponds to E. coli positions 178-194)	CTA GCC/t TTG TAG TTT CAA ACG C (corresponds to E. coli positions 637-658)	Kowalchuk et al. (1997)

*Forward primers are synthesized with a "GC clamp" on the 5' end. The GC clamp is 38 nucleotides long and consists of mostly guanines and cytosines (Gs and Cs). The purpose of the clamp is to improve the resolution of PCR fragments in denaturing gradient gel electrophoresis. The sequence of the GC clamp is 5'- CGC CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGG GG - 3'.

2.5 Statistical analysis.

2.5.1 Principal Component Analysis (PCA) and Redundancy Analysis (RDA).

PLFA fingerprints were analyzed with CANOCO software from Microcomputer Power, Inc. (Ithaca, N.Y.). Mole percents of individual fatty acids were used in the analyses. Both PCA and RDA were used to analyze the data. Whereas PCA is useful for discerning patterns within the PLFA data itself, RDA can be used to test hypotheses regarding the importance of external variables in explaining variation in PLFA data (ter Braak, 1987). PCA describes the axes of maximum variability in the multivariate data set and relationships between environmental variables and the multivariate data can only be quantified indirectly through regression of environmental gradients on ordination axes. RDA is a constrained ordination technique based on PCA, in which ordination axes are constrained to be linear combinations of environmental variables (ter Braak, 1987)) to assess the relationship between environmental variables and the multivariate data. Thus RDA allows direct assessment of the relationship between known

environmental variables and variation in the multivariate data and the significance of the relationship can be tested with the Monte Carlo permutation test.

Environmental variables analyzed with RDA included management regime, sample dates, field blocks, soil texture, and crop. Measures of the microbial community and soil properties, including microbial biomass carbon and nitrogen, substrate-induced respiration, basal respiration, potentially mineralizable nitrogen, soil nitrate and ammonium, and soil moisture content were also analyzed. In the CANOCO program, covariates can be included and thus field level spatial variability was accounted for by using field block as a covariate, except when it was tested as an environmental variable. Sample date and management regime were also included as covariables as noted. The Monte Carlo permutation test (ter Braak, 1990) was used to test the statistical significance of the relationship between environmental variables and variation in PLFA fingerprints. RDA results are displayed on biplots in which relationships among environmental variables and either treatment plots or individual fatty acids are displayed. Environmental variables in RDA can be either continuous (e.g., respiration rate or microbial biomass C) or in the form of categories . Variables which are categories, in this case management regime and sample date, are properly displayed as centroids (ter Braak, 1987) rather than, as for continuous variables, as additional axes on the biplots. Polygons surrounding the same treatments were drawn on biplots to facilitate interpretation. When noted, axes of biplots have been scaled so that their length represents the relative importance of each axis in terms of the percent of the total variation in PLFA fingerprints represented by the axis.

2.5.2 Pattern recognition and neural net analyses.

A data set consisting of nanomole percent values of 26 PLFAs from 245 soil samples was sent to Dr. Phil Hopke, Clarkson University Department of Chemistry. This data set was analyzed with SIMCA (Soft Independent Modeling of Class Analogy), ART-2a (Adaptive Resonance Theory-based neural network), Kohonen neural network; fuzzy ARTMAP, (ART predictive mapping using fuzzy logic), and BP-NN (back-propagation neural network.) The main goal of these analyses was to differentiate samples based on their crop types.

3.0 PLFA RESULTS AND DISCUSSION

A major objective of the first phase of research was to determine if potential source soils collected at numerous locations throughout California could be distinguished based on their PLFA fingerprints. Fig. 3-1 shows an overview of the conceptual framework used to guide this research. We decided it was important for regulatory purposes to have a solid understanding of the basis for differences observed among fingerprints from different soils. Without this basis, it would be easy to argue against a given fingerprint used to identify a particular, for example tomato, field as a source of PM-10. In contrast, knowing that a fingerprint has components representative of tomatoes in general would strengthen its validity as a fingerprint for that particular soil. Also, knowing which PLFAs were responsible for a particular fingerprint could be useful in identifying specific markers for certain soils. Thus, our approach was to ask two main

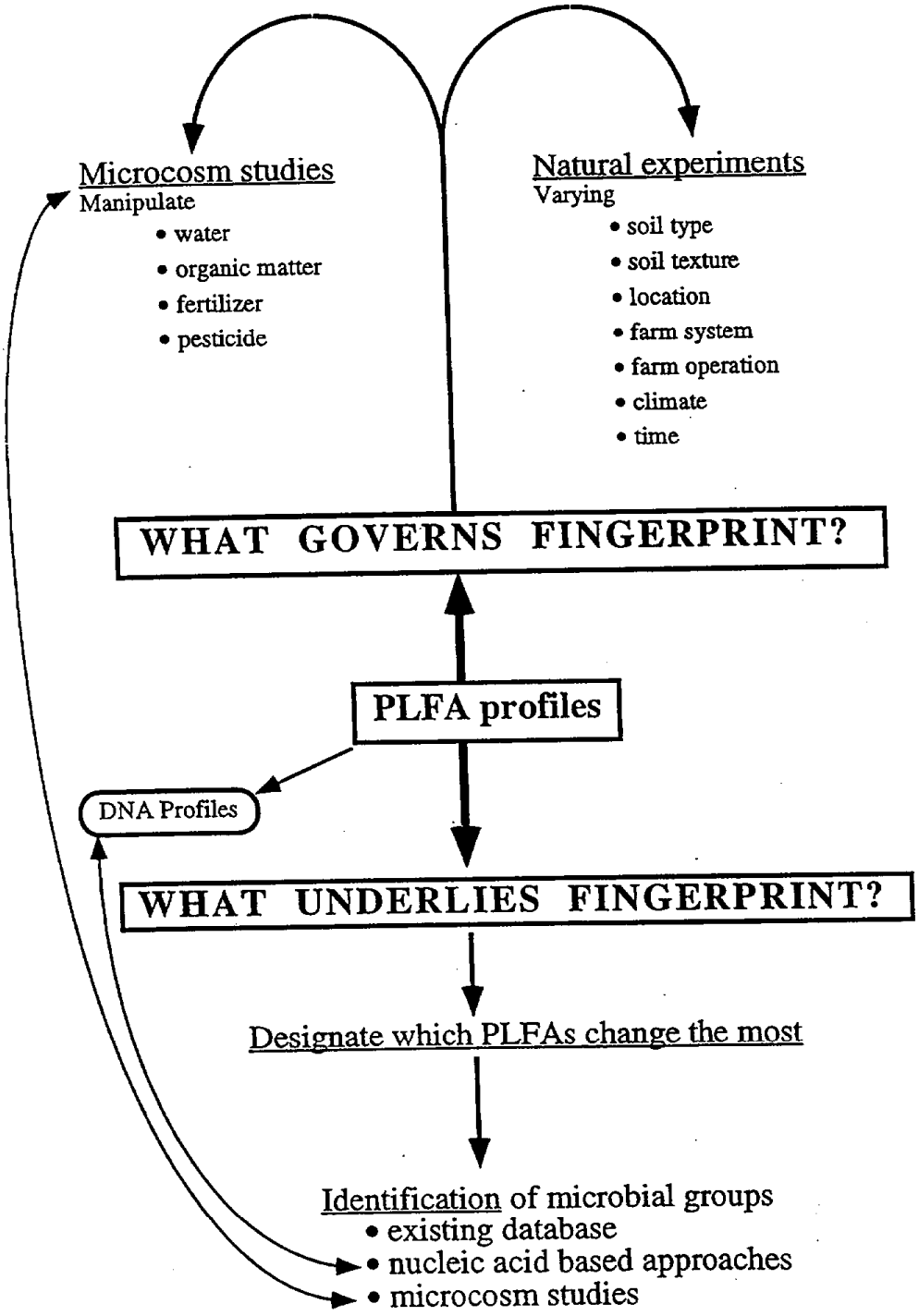

Fig. 3-1. Conceptual diagram of research approach for determining the environmental factors governing and the particular organisms contributing to PLFA fingerprints of sources of airborne dust. Nucleic acid-based (e.g., analysis of DNA) methods fit into the overall approach as a means for identifying the particular species or groups of organisms contributing to the PLFA profiles.

questions: What influences the fingerprint and what lipids are responsible for the particular pattern of a fingerprint?

To provide a better understanding of the foundation for these fingerprints, more in-depth analyses were carried out on subsets of these soils. Thus, the second set of objectives was to determine: a) whether a given fingerprint is consistent for a particular soil at different times and locations within a field and under different management regimes, and b) how crop influences a fingerprint on the same soil. These studies were carried out on a single soil type at university research station field plots near UC Davis. A third objective was to determine how strongly crop and soil texture are related to a particular fingerprint. These studies were carried out at numerous locations on growers fields in the San Joaquin Valley. The fourth objective was to compare PLFA fingerprints of dust and their source soils. These studies were conducted using soil from agricultural fields in the northern and southern Central Valley.

3.1. Analysis of soils from locations throughout California

One hundred fifty soil samples were collected from numerous locations within the California San Joaquin valley. Samples were chosen to represent major crops within the valley, including crops for which the associated management practices are known to produce dust. Phospholipid fatty acid analyses were performed on the samples and a total of 28 lipids were included in the analysis. Using principal component analysis (PCA), the relationships among the different samples were defined. Fig. 3-2 shows that the first and second components explained 32% and 19%, respectively, of the variance in the data. Each point on the graph represents an individual soil sample. Cotton fell on the right hand side of the graph, almonds on the left, and figs, walnuts and tomatoes near the center. Soils from the lower San Joaquin valley, called the ARB soils (including cotton, almonds, walnuts and figs), were distributed broadly within the top half of the graph, whereas soils from the vicinity of Davis (tomatoes, corn, safflower, beans, wheat), in the west Valley near Fresno (tomatoes, cotton), and near Ventura (tomatoes), fell in the bottom half of the graph. The Davis, west Valley, and Ventura soils were relatively similar to one another, whereas the ARB soils were more widely distributed.

Fig. 3-3 shows a plot of the distribution of specific PLFAs that were associated with the distribution of soils shown in Fig. 3-2. This type of plot gives information about which PLFAs were most significant in determining the relationships among the different soils samples. Specific patterns included a greater relative abundance of the fungal marker (18:2) on the left side of the graph (e.g., associated with almonds), greater relative abundance of the actinomycete markers (10Me18:0 and 10Me16:0) on the right side of the graph (associated with cotton), and a split between saturated and unsaturated fatty acids on the right and left side of the graph, respectively.

The soil samples described above, all collected from well-drained agricultural fields, were compared to a broader set of soils which encompassed a greater range of physical and chemical properties. This larger set of soils, which included samples from

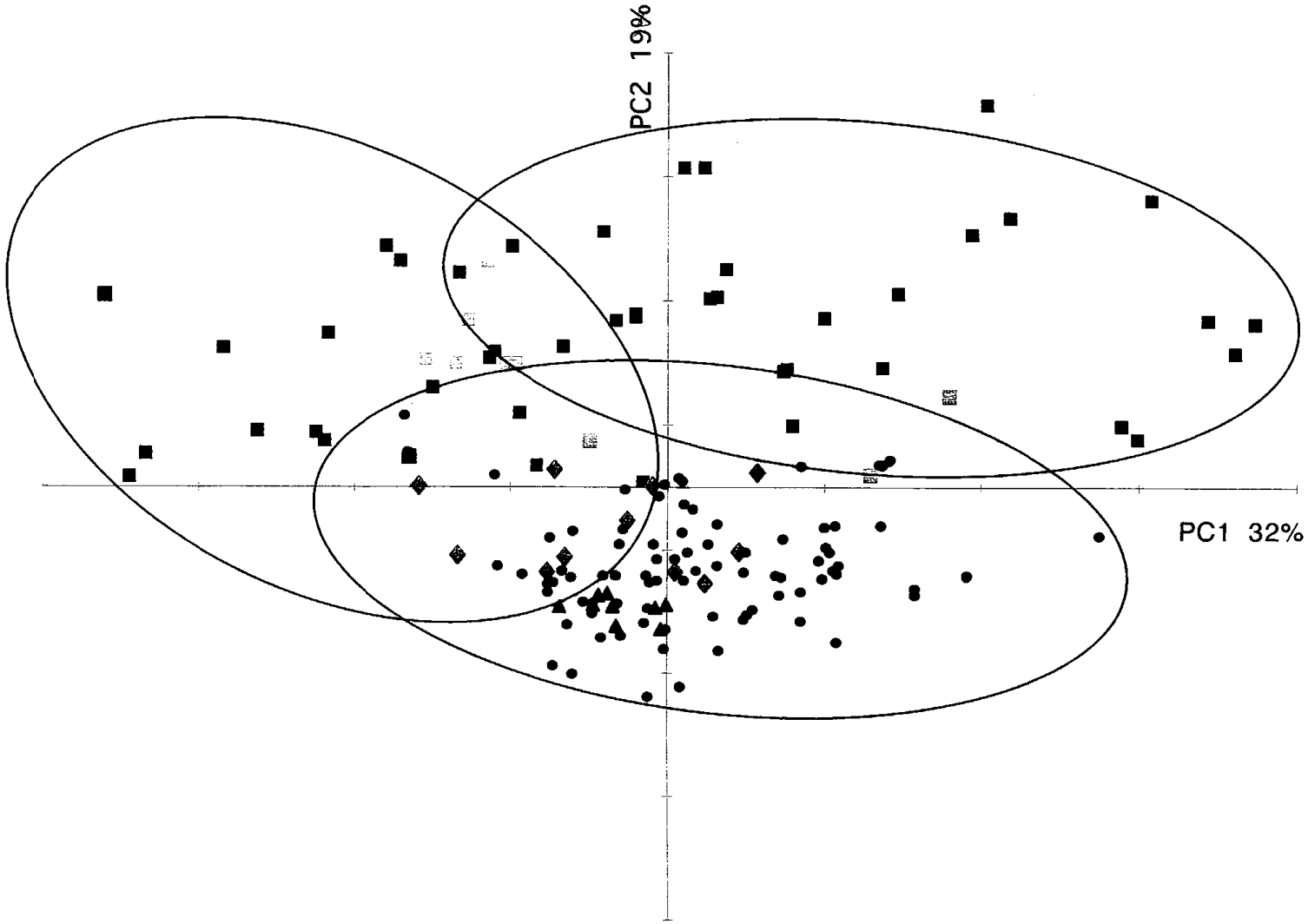

Figure 3-2. Principal Component Analysis plot of PLFA fingerprints of 150 samples of well-drained California agricultural soils representing different crops, management systems, locations and times of year. ARB soils from the lower San Joaquin Valley (squares) were distributed broadly within the top half of the graph, with cotton (light green) on the right, almonds (dark blue) on the left, and figs (yellow) and walnuts (lavender), distributed more in the center. Soils from the vicinity of Davis (tomatoes, corn, safflower, beans, and wheat , all coded as red circles), soils from the west Valley near Fresno (tomatoes, cotton, coded in green triangles), and soils near Ventura (light blue diamonds) fell within the bottom half of the graph. The Davis, west Valley, and Ventura sols were relatively similar to one another, whearas the ARB soils were more widely distributed.

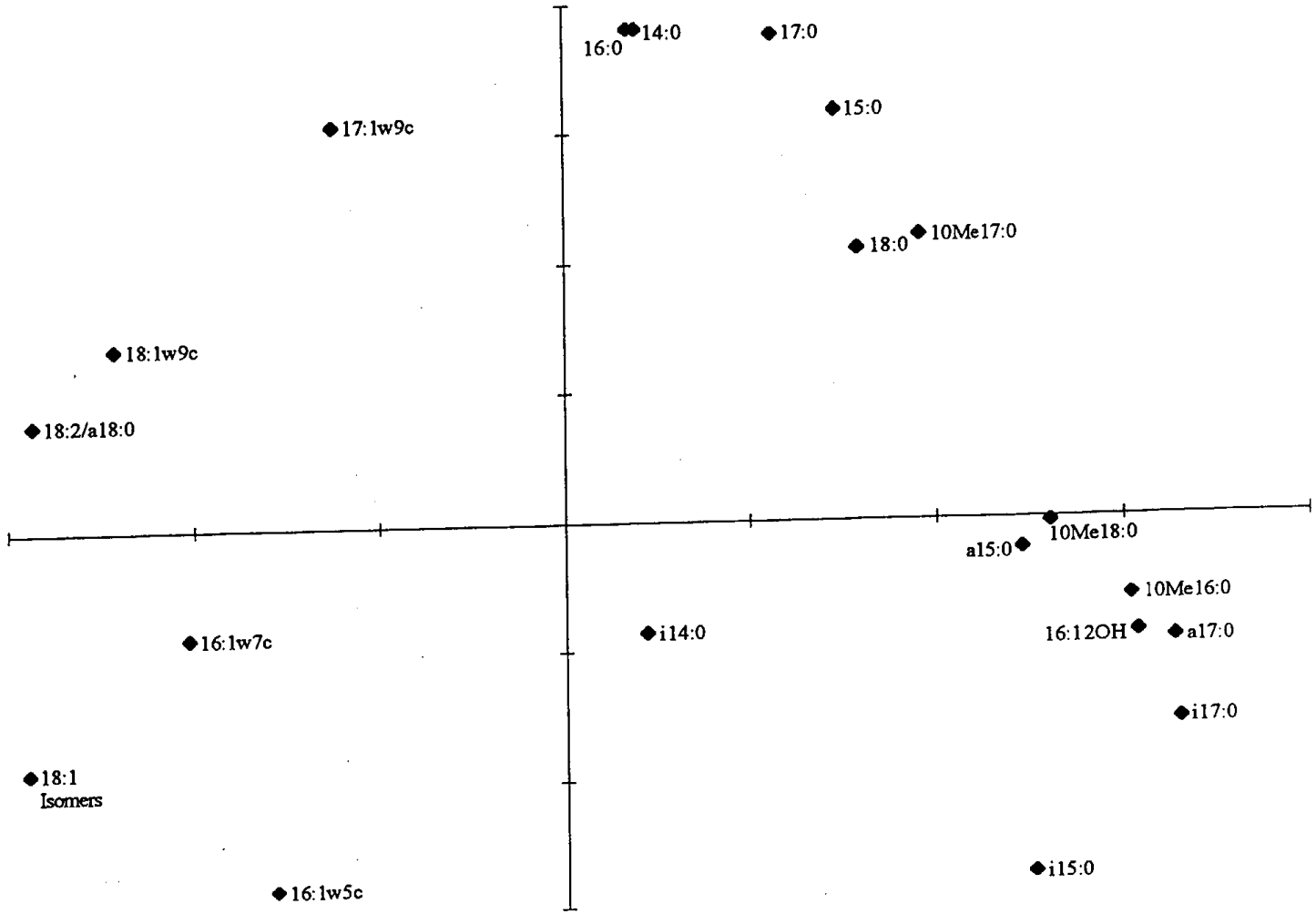

Fig. 3-3. Plot showing the fatty acid loadings in the first two principal components in Fig. 3-2. The location of each PLFA correlates with the position of the samples shown in Fig. 3-2.

rice fields during the winter fallow, Clear Lake sediment, and high organic matter soil from the Delta region, were reanalyzed by PCA. This new group of samples shared the property of being water saturated or poorly drained. In this PCA (Fig. 3-4), there was a strong separation on the first axis between one group consisting of rice soils, Delta soils and lake sediment and another group consisting of the San Joaquin valley soils described in Fig. 3-1. The clay soils cropped with cotton fell at an intermediate point, on the first axis, between the San Joaquin soils and the poorly drained soils. However, the patterns described in Fig. 3-2 could still be discerned in Fig. 3-4 within the San Joaquin valley soils.

The basis for the differences in PLFA fingerprints was further explored in specific subsets of the soil samples described above. We undertook this analysis to help determine how robust a fingerprint was for a given soil, if it was possible to develop predictive relationships between fingerprints and soil properties, and to help in quality control analyses of the analytical method.

3.2 Sustainable Agriculture Farming Systems (SAFS) soils.

A detailed analysis was conducted of PLFA fingerprints in soils collected from the Sustainable Agriculture Farming Systems (SAFS) plots at UC Davis. These plots are representative of farming practices typical to the Sacramento Valley and provide an opportunity to measure the importance of environmental and management variables on PLFA fingerprints on the same soil type. The main objectives were to test whether there was consistency in the fingerprint of a given field throughout the season, at different spatial locations within the field, and under different farming management practices. This research was co-funded by a grant from the USDA National Research Initiative. These results are summarized in Bossio et al. (1997).

3.2.1. Effect of spatial variability and management system for the same soil and crop.

PLFA fingerprints were consistent among field blocks within the same farming system, thus field variability represented by blocks in this study did not have a significant impact on the differences in the observed fingerprints. Management regime, however, did influence PLFA fingerprints. Organic and conventionally managed plots were significantly different ($p<0.05$) from each other on all sample dates, except July 3 when conventional plots were less different from the other two systems ($p<0.10$). The low input plots were not different from organic except on May 23, and low input was not significantly different from conventional plots except on April 4 and April 18. The amount of variation in PLFA data explained by the first two RDA axes with management regimes as the only environmental variables ranged from a low of 34% on May 23 to over 50% on Apr. 18, May 9, Jul. 3, and Jul. 28.

PLFA data were related to other more conventional measurements characterizing microbial populations by including these other measurements as environmental variables in a redundancy analysis (RDA) and testing for significant relationships with the Monte Carlo permutation test. Microbial biomass carbon and nitrogen, substrate induced

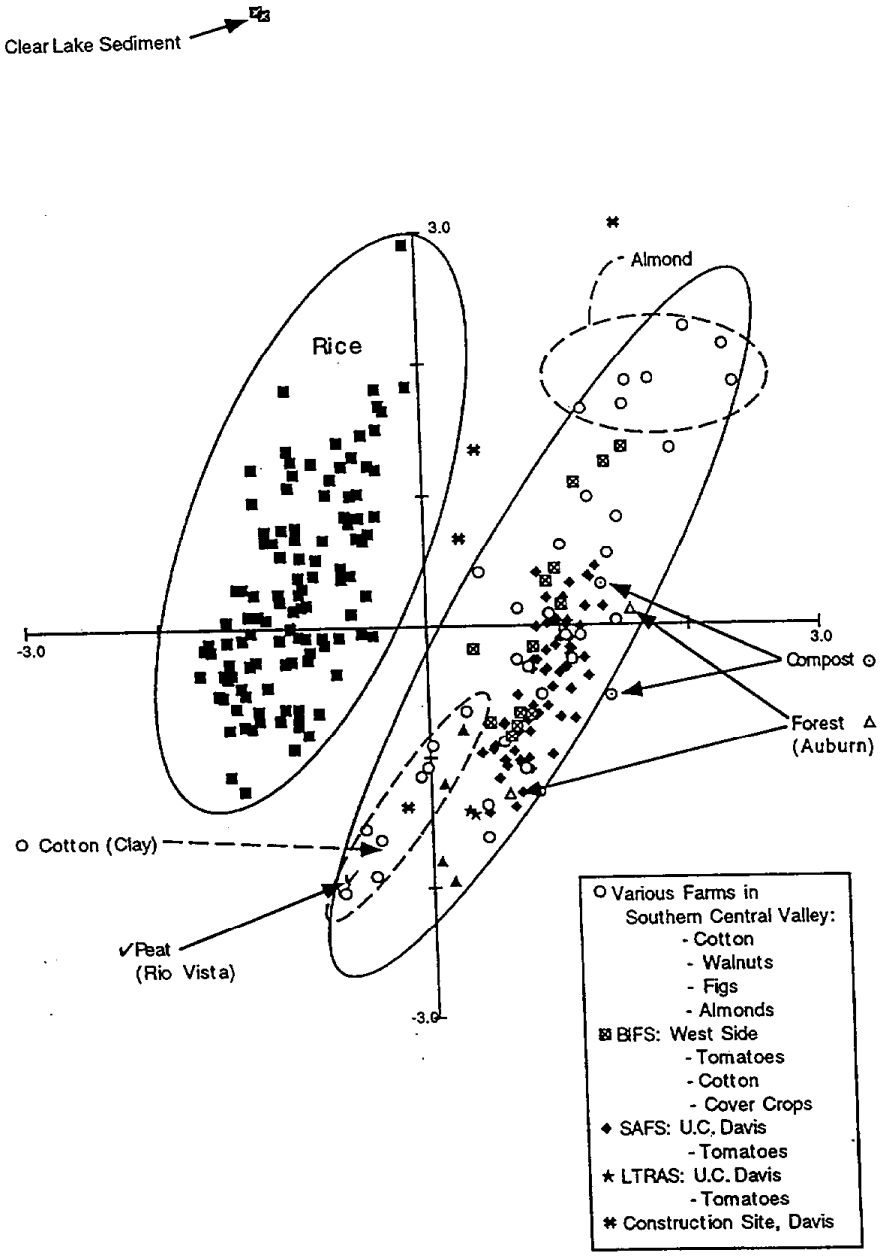

Fig. 3-4. Principal component plot of PLFA fingerprints of samples of both well-drained and flooded agricultural soils and compost, as well as lake sediment, from California. The figure includes the soils typical of the California Central Valley (previously shown in Fig. 2) and shows how they relate to a broader class of soils and sediment. The rice soils, which vary in management, time of sampling, group together in the left side of the graph, whereas all the well-drained agricultural soils group together on the right side. Sediment, peat soils, and clay soils are grouped with the rice soils on the first axis, but are separated along the second axis. Compost and forest soils group with the well-drained soils but fall on the right side of the grouping. The differences among the valley soils visible in Fig. 2 are still evident even within a very diverse group of soils.

respiration, basal respiration, potentially mineralizable nitrogen, soil nitrate and ammonium levels, and soil moisture content were not consistently associated with PLFA fingerprints. Soil respiration rates were associated with differences in PLFA fingerprints early in the season but not in July. The fact that microbial biomass measures, (e.g. fumigation extraction, substrate induced respiration) were not associated with PLFA variation was expected, because the importance of biomass is eliminated from the PLFA data by analyzing relative abundances rather than absolute masses of fatty acids.

The specific PLFAs whose relative abundance differed most among farming systems and dates were analyzed in two ways. First, an RDA of all sample dates combined with field blocks and sample dates as covariables gave a composite average response of fatty acids to management treatment. In the composite analysis, each management treatment was significantly different ($p=0.01$), from all other treatments and, as with analysis of separate sample dates, low input fell between organic and conventional on the first ordination axis. On a biplot of fatty acid scores based on this composite analysis, a group of 5 fatty acids were strongly enriched in the organic system, whereas another group of 3 fatty acids had a lower relative abundance in organic plots when compared to conventional plots. An actinomycete indicator was most strongly enriched in the low input plots, and a fungal indicator was enriched in both organic and low input plots.

3.2.2 Effect of season within one year for the same soil and crop.

Differences in PLFA fingerprints among sample dates were highly significant ($p<0.01$) across all dates. Sample dates separated along a continuum on the first axis (37% of the variation) in RDA in order of their occurrence in time (Fig. 3-5). To look at sample dates as a single source of variation in this analysis, both field blocks and treatments were included as covariables. On the biplot, sample date groups are labeled on their centroids, the average position of all the plots from each sample date. When both management treatments and sample dates are included as environmental variables, it is possible to evaluate the relative magnitude of these two variables based on distance of the centroids for these variables on the biplot. The differences between April and July were larger than differences among management regimes. Over shorter time periods, e.g., between early and late May, the difference among sample dates is similar in magnitude to differences due to management. In April, differences among management treatments were larger than differences associated with sample dates that were only two weeks apart.

To determine the importance of season and management on PLFA profiles within one soil/crop type relative to differences in another soil/crop type, the SAFS data set was compared to another large set of PLFA data collected from rice soils (supported by a grant from Ducks Unlimited to study the effects of different methods for management of straw disposal). Different rice straw management methods and winter flooding treatments had been applied in a replicated experiment to this other soil type, a Willows clay. When PLFA profiles from all sample dates and treatment plots of the SAFS plots were compared to the Willows clay soil, the cloud of points representing variation from

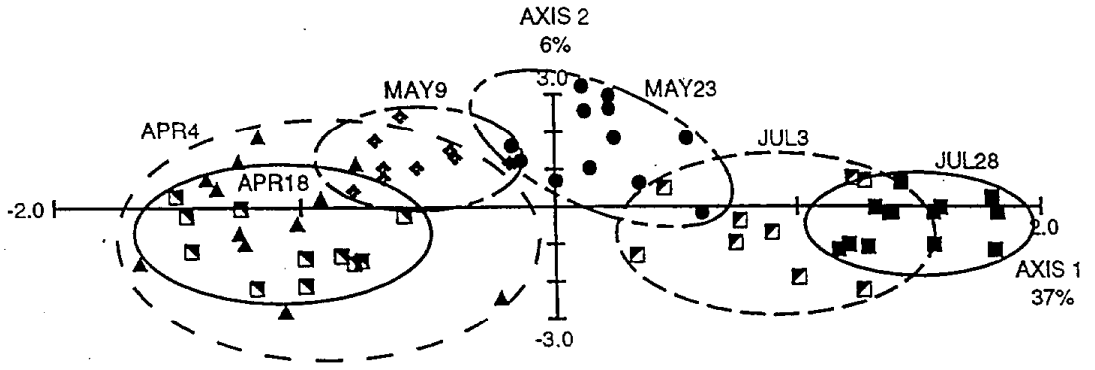

Fig. 3-5. Redundancy analysis plot of PLFA fingerprints of tomato soils collected over the growing season in 1995 at the SAFS plots. The first axis is constrained to time, thus the linear pattern of the changes is strongly correlated with time. Management system could also be distinguished within most of the dates, but had less of an effect on the PLFA fingerprints than did time of season.

season and management for each field experiment were clearly separated from the other soil type on a highly significant axis representing 80% of the variation in PLFA profiles (Fig. 3-6). Most monounsaturated fatty acids, the fungal indicator, and the actinomycete indicator had higher relative abundances in the SAFS soil. The Willows clay had higher relative abundances of most branched fatty acids, most straight chain fatty acids, and other 10 Methyl substituted fatty acids. These results are summarized in Bossio et. al. (1997).

3.2.3 Effect of year for same soil.

Soil samples were collected from the SAFS plots at different times in 1995, 1996 and 1997. A PCA plot of SAFS tomato soils collected over the 3 years (Fig. 3-7) show a relationship with time along the first axis. Samples collected early in the year fall out on the left side of the graph, whereas samples collected later in the season, regardless of year, fall on the right hand side of the graph. Although the July samples were somewhat similar to one another, there were differences between the 1995 and the 1996/97 samples as seen by their separation along the second axis.

Fig. 3-8 shows a PCA plot of data collected for different crops and different times at the SAFS plots. Again a trend with time can be discerned along the first axis. Corn samples collected in 1995 were more similar to 1995 tomatoes than they were to corn samples collected in 1997.

3.2.4 Effect of crop for the same soil.

A comparison was made of PLFA fingerprints of tomato, corn, safflower, bean and wheat soils all collected from the SAFS plot on a single date in the latter part of the growing season in 1997. Though some crops could be distinguished from one another, their influence on the grouping of the soils was not as clear as in the case of the PM-10 soils (Fig. 3-9) where crop appeared to have a stronger influence on the fingerprints than did soil texture.

3.3 PM10 soils.

PCA plots of PLFA fingerprints from 40 soils selected from the UCD Air Quality Group's PM10 Project are shown in Figs. 3-10 and 3-11. These soils had been sampled during the 1994 harvest season, analyzed for particle size distribution, and stored at ambient temperature in the air-dried state. We chose these soils for PLFA analyses because they represented different textural classes and crop types, enabling us to test effects of these variables on PLFA profiles. Fig. 3-10 shows a PCA plot of these soils in an analysis that did not include road samples. Fig. 3-11 shows a PCA plot that includes road samples.

3.3.1 Relationship between crop or soil texture and PLFA fingerprint.

The quantitative relationship between crop or soil texture and PLFA fingerprints was determined in the lower San Joaquin Valley soils in which % sand, % silt, % clay,

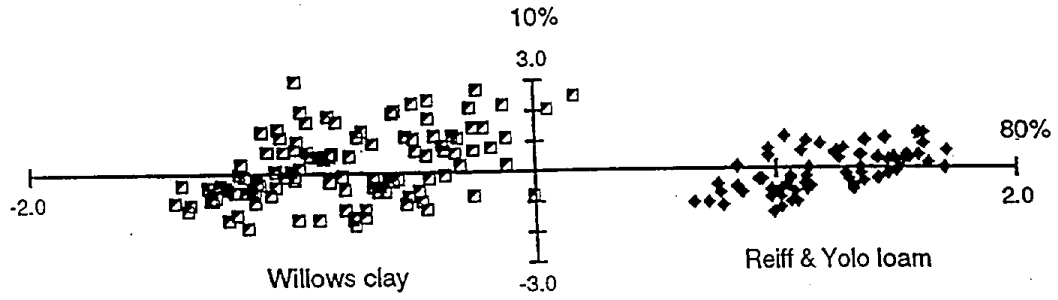

Fig. 3-6. Principal component analysis plot of PLFA fingerprints of all SAFS tomato soils and Maxwell rice soils, including samples representing different management practices and time of sampling. The first axis explains 80% of the variance in the data. These factors contributed to variability of the fingerprint for each soil; however, the soils could be clearly distinguished based on their location.

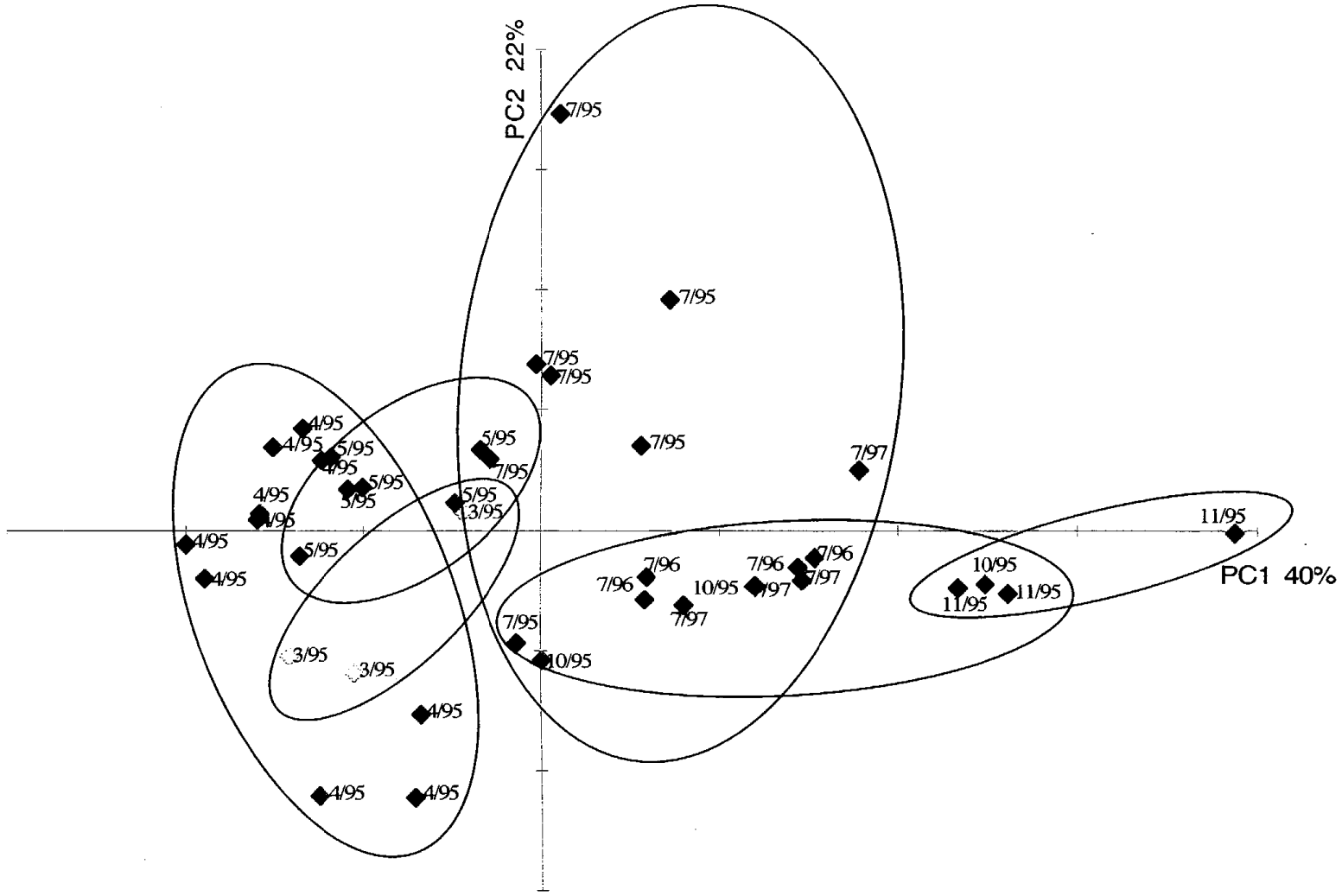

Figure 3-7. Principal Component Analysis plot of PLFA fingerprints of tomato soils sampled on different dates.
Tomato soils sampled early to late in the growing season showed a distribution trend from left to right along the X axis. Color codes are for soils sampled in 3/95 (yellow), 4/95 (pink), 5/95 (blue), 7/95 (red), 10/95 (green), 11/95 (purple). Soils sampled from the same plots in July 1995, 1996, and 1997 (all in red) showed relatively consistent locations relative to the X axis from year to year.

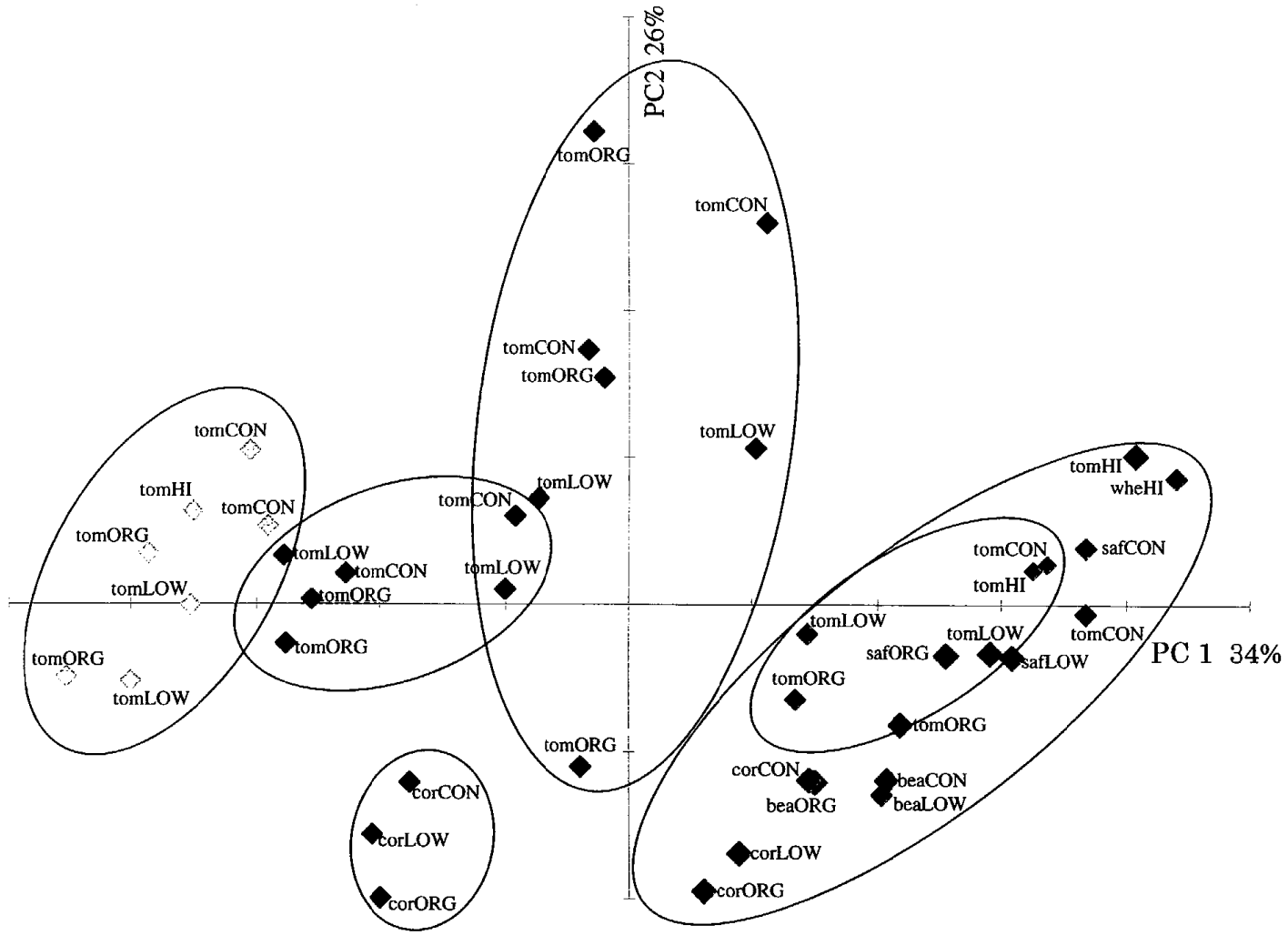

Figure 3-8. Principal Component Analysis plot of PLFA fingerprints of SAFS soils under different management treatments and crops (DIFFERENT YEARS). PLFA fingerprints of soils under conventional (CON) and high-input (HI) management tended to be located higher on the Y axis than fingerprints from soils under organic (ORG) and low-input (LOW) management for most crops and sampling dates. As in Figure 3-7, soils sampled earlier in the season were distributed on the lefthand side of the X axis, while soils sampled later in the season showed a trend toward the right. Color codes are for sampling dates in 4/95 (yellow), 5/95 (pink), 7/95 (blue), 8/95 (red), 7/96 (green), and 7/97 (purple). Crops are tom (tomato), cor (corn), saf (safflower), bea (bean), and whe (wheat).

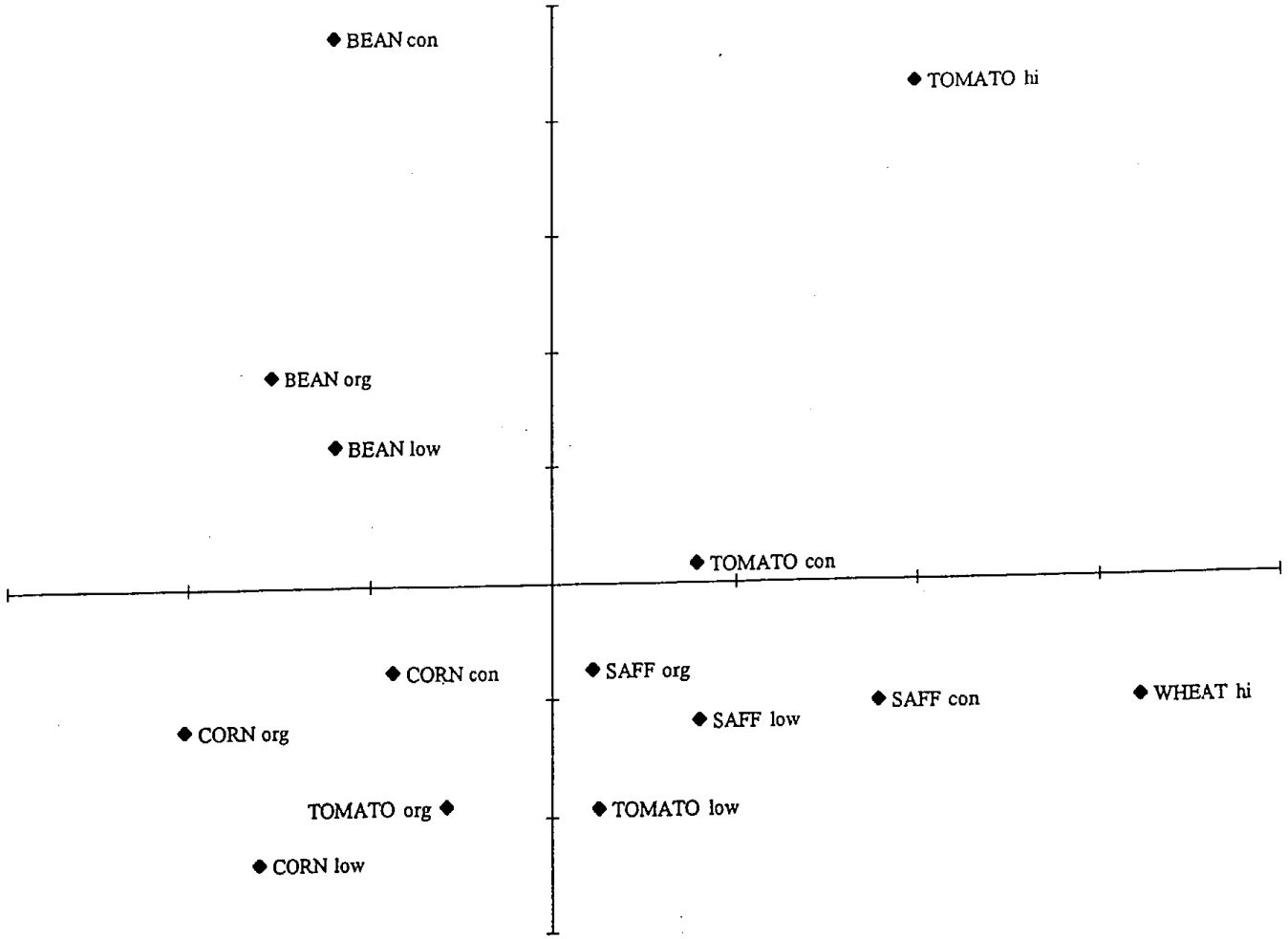

Fig. 3-9. Principal component analysis plot of PLFA fingerprints of corn (cor), tomato (tom), beans (bea), safflower (saf) and wheat (whe) soils cultivated using conventional 4 yr (CON), conventional 2 yr (HIGH), low input (LOW) and organic (ORG) management practices during 1997 at the SAFS plots. This plot removes the potential confounding factor of differences among years from the effect of crop. Though some soils under some crops could be easily distinguished from one another, the effect of crop

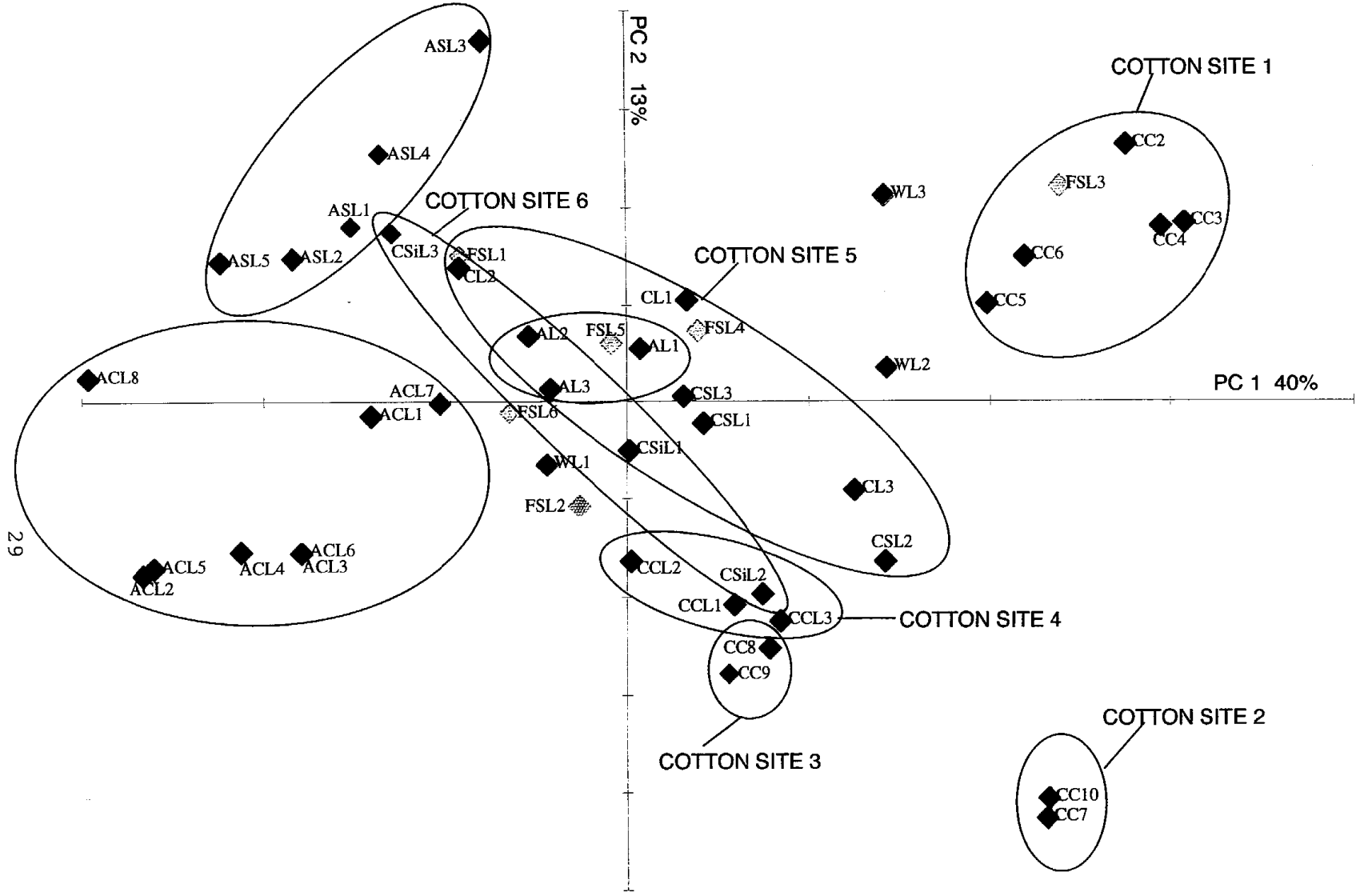

Figure 3-10. Principal Component Analysis plot of PLFA fingerprints of air-dried soils under different crops, soil textures, and locations.
Almond soils (blue) were distributed on the lefthand side of the X axis, cotton soils (red) on the right, while fig (yellow) and walnut soils (purple) were more broadly distributed. Almond soils, which were sampled from one area, tended to cluster by texture. Cotton soils were sampled from six different areas, and cotton soils of similar textures clustered by sampling area. The first letter in each label represents crop (A = almond, C = cotton, F = fig, W = walnut); second letters represent soil texture (SL = sandy loam, L = loam, SiL = silt loam, CL = clay loam, C = clay); the number in each label represents location within field.

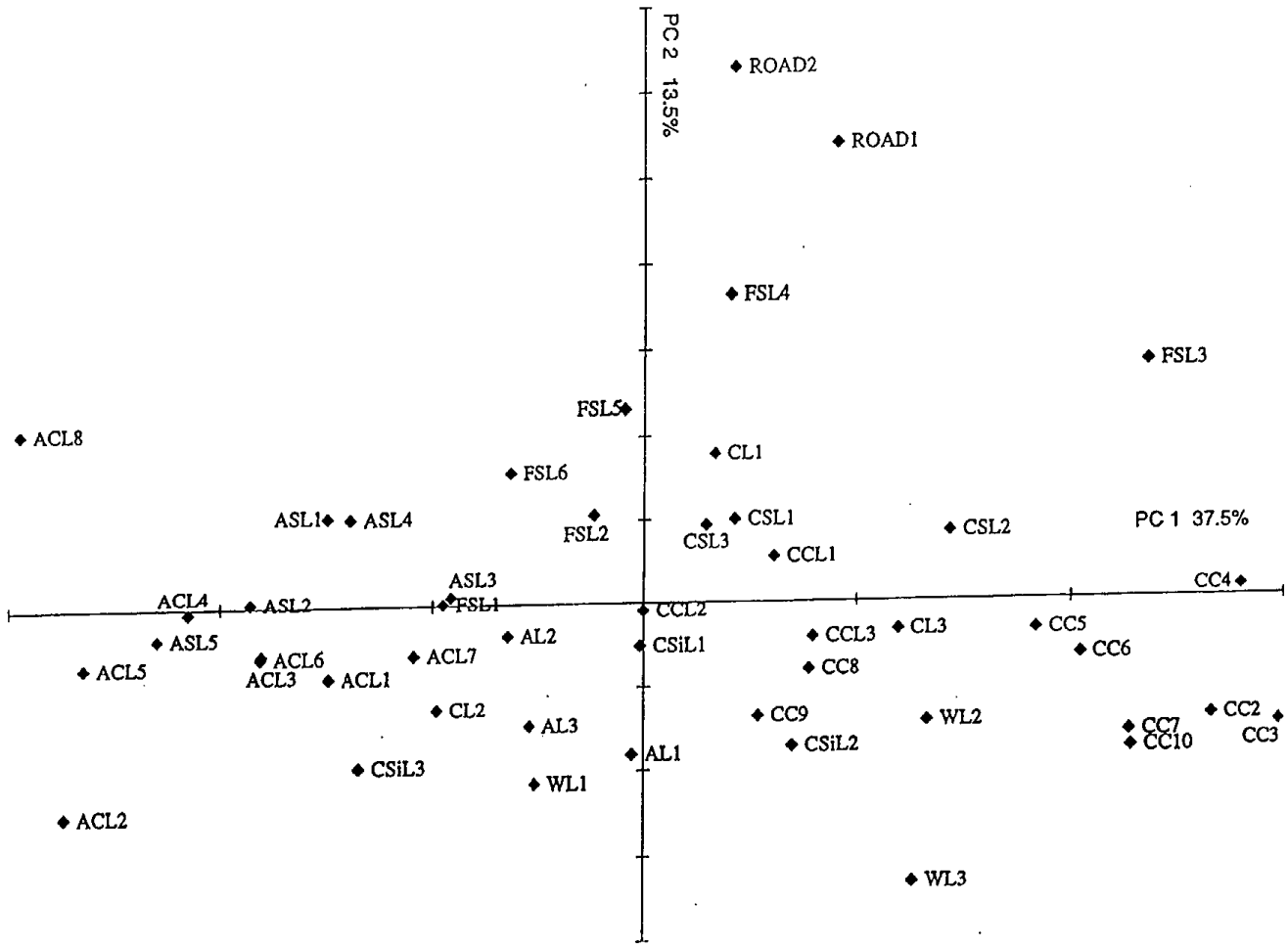

Fig. 3-11. Principal component analysis plot of PLFA fingerprints of the PM-10 soil samples. These samples were collected from the lower San Joaquin Valley and are part of a USDA-funded project to determine potential soil sources of fugitive dust. This plot includes samples from both the agricultural plots and adjacent unpaved roads.

and crop type were measured. Whereas PCA visualizes the variation in the data in relation to the best fitting theoretical variables (components) made up of individual PLFAs, redundancy analysis (RDA) directly relates the variation in PLFA data to specific environmental variables. RDA revealed that both soil texture (percentage of clay, sand or silt) and crop type were significantly related with the variation in the PLFA profiles across soils (Fig. 3-12). The diagonal axes represent the gradients of each soil particle and the crop names represent centroids. The distance of the points (representing soil samples) to either the diagonal axes or centroids represent how strongly these variables were correlated with the variation in the PLFA data for each sample. Overall, crop type was a stronger determinant of PLFA profile than soil texture. Almond and cotton soils were clearly segregated on the basis of the two principal components. Walnut and fig soils appeared to have PLFA profiles that were intermediate between those of almond and cotton soils, although more of these samples would need to be analyzed to verify their distribution patterns. Finer-textured soils (with higher clay contents) were clearly segregated from medium-textured (loam) soils associated with the same crop. Medium-textured cotton soils clustered together more tightly than did the medium-textured almond soils. Trends in the loadings of specific PLFAs included a high relative abundance of markers for fungi and gram negative aerobic bacteria along the sand axis, a low relative abundance of saturated PLFAs along the silt axis, and a high relative abundance of markers for gram positive (usually anaerobic) bacteria and actinomycetes along the clay axis.

3.4 Quality assurance considerations.

Total PLFA yields were determined in triplicate for seven PM_{10} soils of varying textures during a quality assurance evaluation of PLFA extraction consistency. Total PLFA yields ranged from 130 to 900 nanograms per gram of dry soil. The source of greatest variability in total PLFA yields within triplicate samples appeared to be contaminants that eluted from the chromatography column after fatty acid 20:0. (This fatty acid is the last one identified by the Sherlock Microbial Identification System, or MIS. Peaks before 20:0 are identified by MIS and assigned fatty acid names. Peaks after 20:0 are not assigned names by MIS, but they provide additional data which we could incorporate into more comprehensive fingerprints.) We detected contamination by comparing the sums of PLFA peak areas before and after 20:0 across each set of triplicate samples.

Tables 3-1a and 3-1b give the mean PLFA yields for the 7 soils before and after 20:0, respectively. For yields before 20:0, coefficients of variation (CVs) ranged from 2 to 11% (except for the cotton clay loam soil, which had a CV of 39%.) For yields after 20:0, most CVs were much higher, indicating that the size of this fraction was much more variable. This variability appeared to be due to contamination by larger compounds that remained in the chromatography column from previous samples. Figures 3-13a and 3-13b show the locations of fatty acid 20:0 in two PLFA profiles from replicate samples of the same soil. In Figure 3-13b, the peaks after 20:0 are more numerous and higher than those in Figure 3-13a. It appears that the PLFA extract which produced the profile in Figure 3-13b was contaminated, while the extract corresponding to Figure 3-13a was not.

Table 3-1a. Ranges, means, and standard deviations of PLFA yields measured by the gas chromatograph before fatty acid 20:0

Soil	Range of PLFA yields (ng per gram dry soil)	Mean PLFA yield (± one standard deviation)	Coefficient of variation (percent)
Clay (Location 1, cotton)	159-188	172 (± 15)	8
Clay (Location 2, cotton)	162-182	167 (± 13)	8
Clay loam (Cotton)	144-328	236 (± 92)	39
Silt loam (Cotton)	169-210	189 (± 21)	11
Loam (Cotton)	216-231	224 (± 8)	3
Sandy loam (Cotton)	102-124	111 (± 12)	11
Sandy loam (Almond)	590-659	632 (± 37)	6

Table 3-1b. Ranges, means, and standard deviations of PLFA yields measured by the gas chromatograph after fatty acid 20:0

Soil	Range of PLFA yields (ng per gram dry soil)	Mean PLFA yield (± one standard deviation)	Coefficient of variation (percent)
Clay (Location 1, cotton)	38-185	90 (± 83)	92
Clay (Location 2, cotton)	17-88	44 (± 38)	85
Clay loam (Cotton)	29-315	139 (± 154)	111
Silt loam (Cotton)	21-40	31 (± 10)	31
Loam (Cotton)	34-38	37 (± 3)	7
Sandy loam (Cotton)	23-84	44 (± 35)	79
Sandy loam (Almond)	148-266	208 (± 59)	28

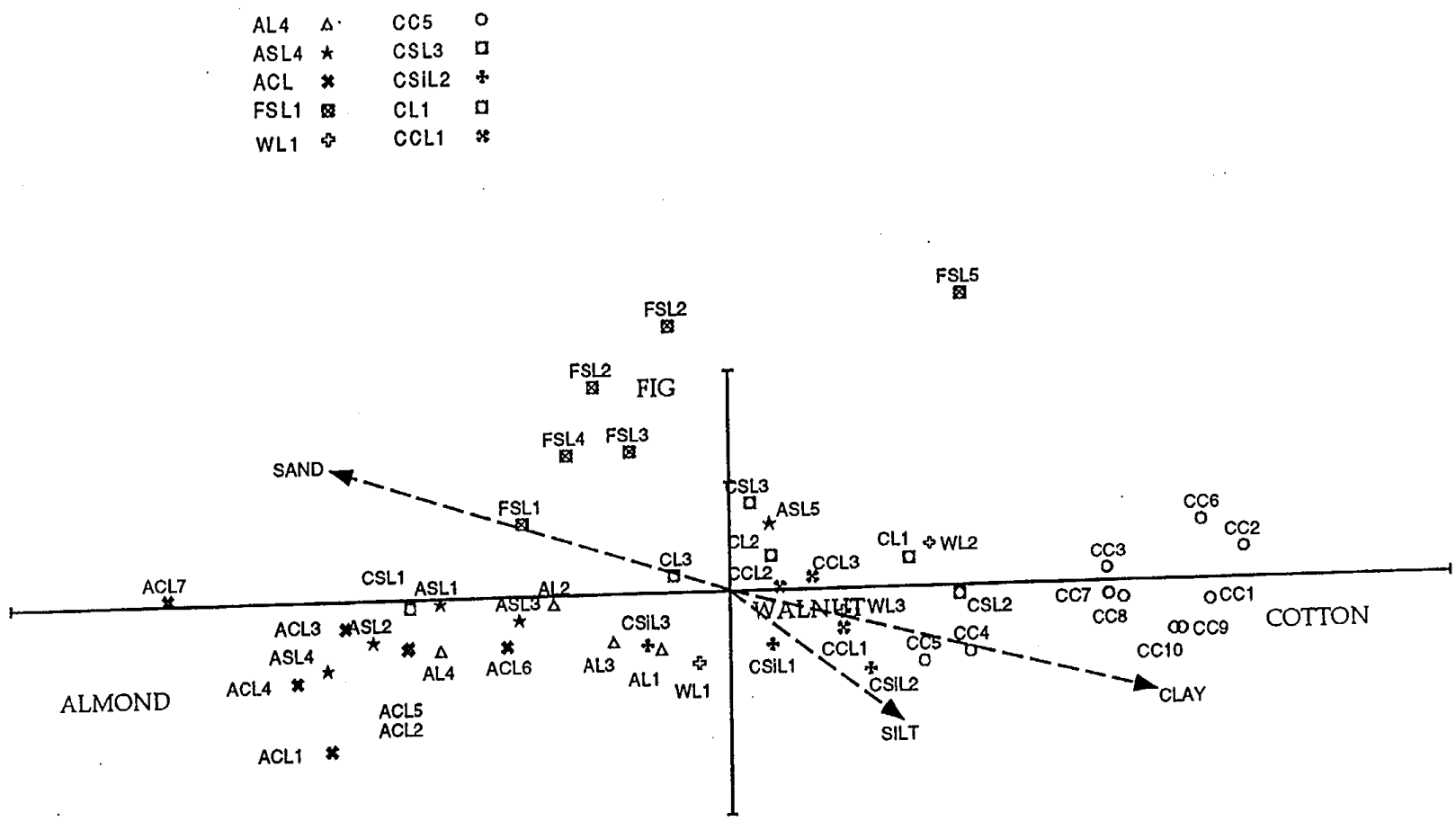

Fig. 3-12. Redundancy Analysis Plot of PLFA fingerprints of PM-10 soil samples. The crop type, percent sand, silt and clay are included in this analysis to demonstrate the effect of these environmental variables on the distribution of samples. Crop and texture are plotted with the samples to show the correlations between the PLFA fingerprint for each sample and the crop type and soil texture. Monte Carlo permutation tests show that crop and soil texture significantly affect the distribution of samples.

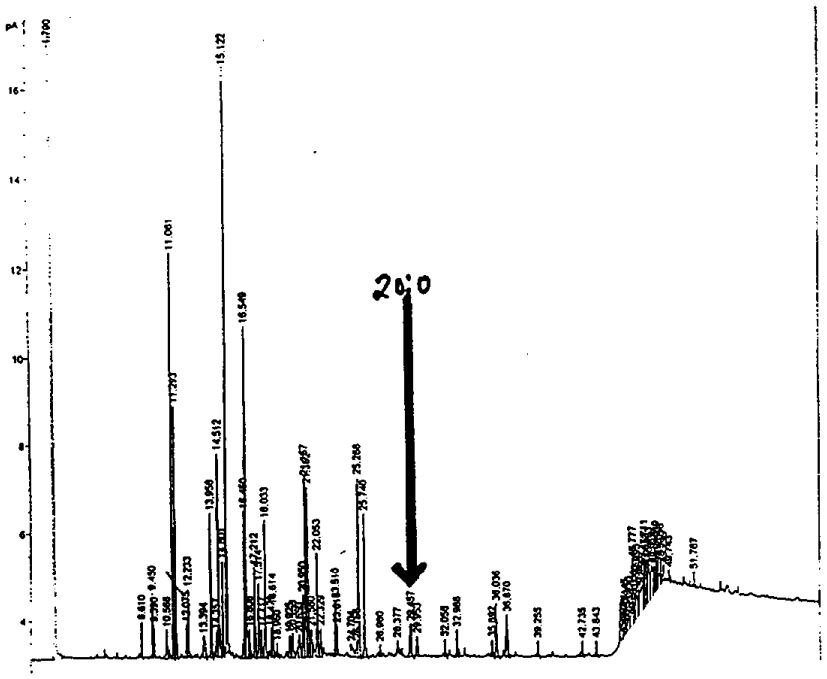

Fig. 3-13a. Gas chromatogram of typical sample showing position of last fatty acid identified in each sample, and normal amount of unidentified compounds.

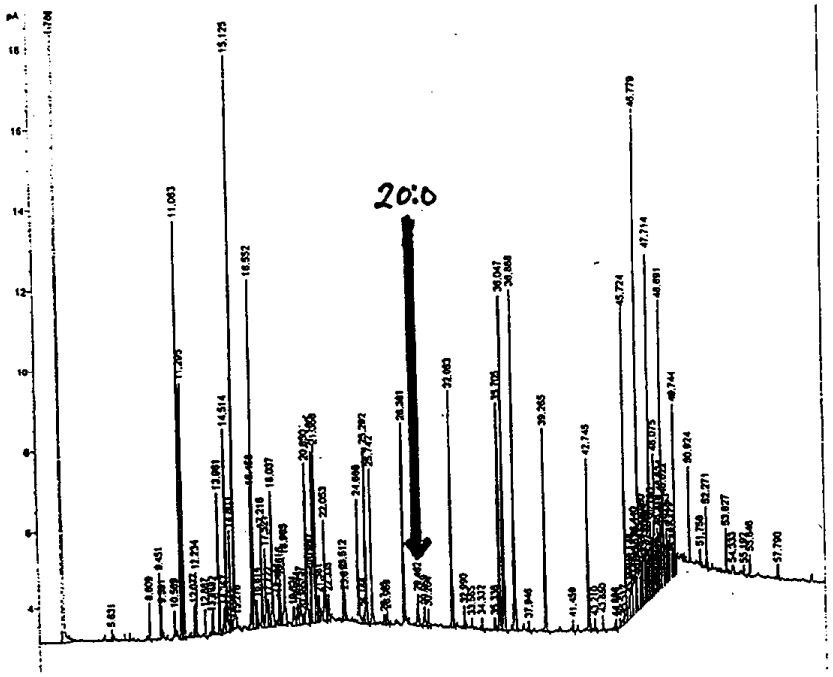

Fig 3-13b. Gas chromatogram of contaminated sample. The peaks to the right of the 20:0 fatty acid are unidentified contaminants.

Since contamination peaks might interfere with the naming of fatty acids that come off the column prior to 20:0, we are evaluating the relative positions of contaminated samples in PCA plots. We have also reduced the incidence of contamination fourfold by stoppering the sample vials with a different type of septa and changing the column liner more frequently.

Large, "contaminant" peaks occurring after fatty acid 20:0 were fatty acid 20:0 were mainly responsible for the high variability in total PLFA yields among triplicate lab samples. These peaks were typically present at low, background levels in PLFA profiles from soils, but we did not observe them in negative controls (test reagents and water). These peaks did not match those produced by dissolved septa material, which we had suspected to be a possible contaminant. We reduced the incidence of these peaks from 15% to less than 4% of samples tested by changing the gas chromatography (GC) standards, liners, and septa more frequently. Since we were not able to completely eliminate these peaks, we analyzed these peaks on Dr. Rick Higashi's GC-MS. He found that they were long-chain aliphatic compounds, which could be septa bleed or injector port liner residues. We addressed this by changing the solvent rinse vial more frequently and increasing the number of syringe rinses between injections.

3.5 Comparison of PLFA fingerprints of dust and soil.

Principal component analyses were performed on PLFA fingerprints of paired dust and soil samples from: i) the Campbell tract (CT) near Davis, CA, and ii) Westhaven soil (WW), Kimberlina soil (KW), and a composite dust sample (WK) at the Stone Land (SL) site near Coalinga (Fig. 3-14). The first and second components represent 59 and 27%, respectively, of the variance in the data. The dust sample from the Stone Land site was distinctly different from its potential source soils, whereas the Campbell tract dust sample was very similar to its potential source soil. Evaluation of the plot of PLFA loadings for the principle component analysis plot shown in Fig. 3-15 revealed that high relative abundances of the PLFAs located in the left hand side of the graph were responsible for strong separation of the Stone Land dust sample (WK) from its potential source soils. These PLFAs included 14:0, 16:0, 18:0, 20:0, and 18:1w9c. These particular PLFAs are present in high amounts in plant cellular material and are not as abundant (dominant) in microbial tissues.

To assess how much the plant-derived material contributed to the separation of the Stone Land dust and soil samples, another principle component analysis was performed on PLFA fingerprints, this time excluding those PLFAs associated with plant material (Fig. 3-16). The first and second components represent 62 and 19%, respectively, of the variance in the data. In this case, the differences between the Stone Land soils and dust samples were not as great as when the plant associated PLFAs were included in the analysis (e.g., Fig. 3-14).

These results suggested that the Stone Land dust samples were substantially enriched in plant material to levels far in excess of what was present in their source soils. This type of enrichment was not universal among dust samples as indicated by the lack of

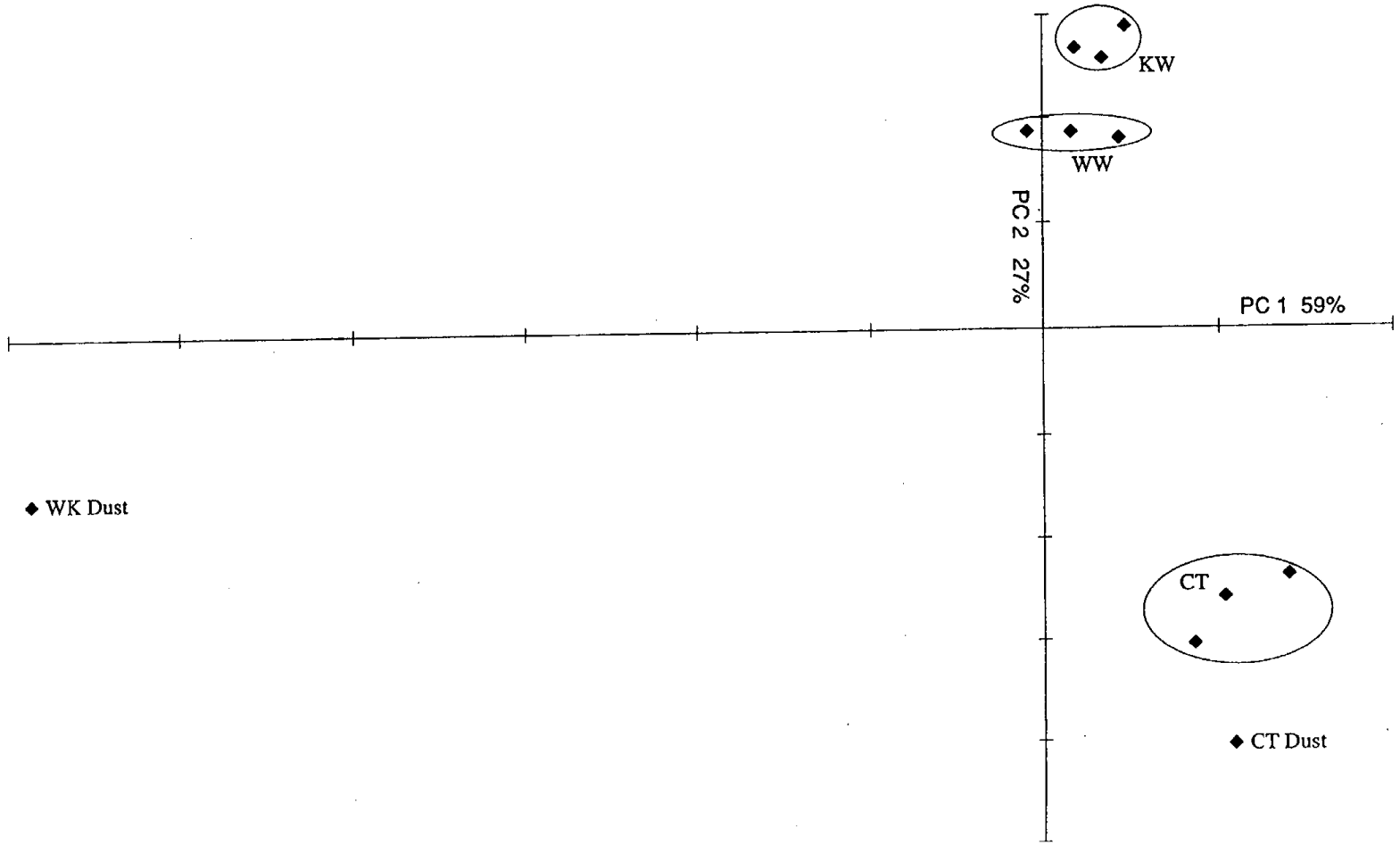

Fig. 3-14. Principal component analysis plot of PLFA fingerprints of paired dust and soil samples from: i) the Campbell tract (CT) near Davis, CA, and ii) Westhaven soil (WW), Kimberlina soil (KW), and a composite dust sample (WK) from Stoneland, CA. The first and second components represent 59 and 27%, respectively, of the variance in the data. The dust sample from Stoneland was distinctly different from its potential source soils, whereas the Campbell tract dust sample was very similar to its potential source soil.

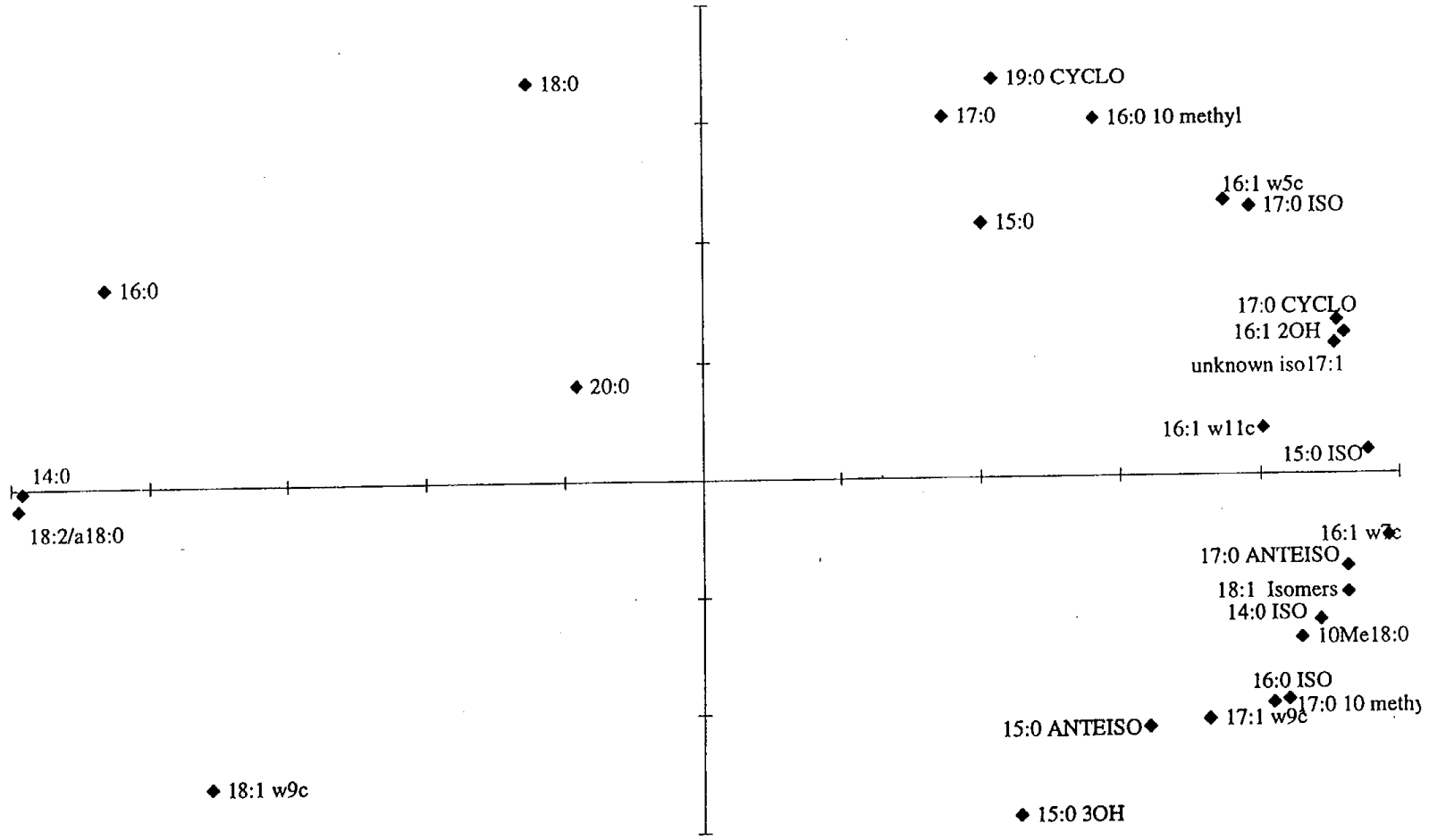

Fig. 3-15. Plot of PLFA loadings for the principal component analysis plot shown in Fig. 3-14. High relative abundances of the PLFAs located in the left hand side of the graph were responsible for strong separation of the Stoneland dust sample (WK) from its potential source soils. These PLFAs are present in high amounts in plant cellular material and are not abundant in microbial tissues.

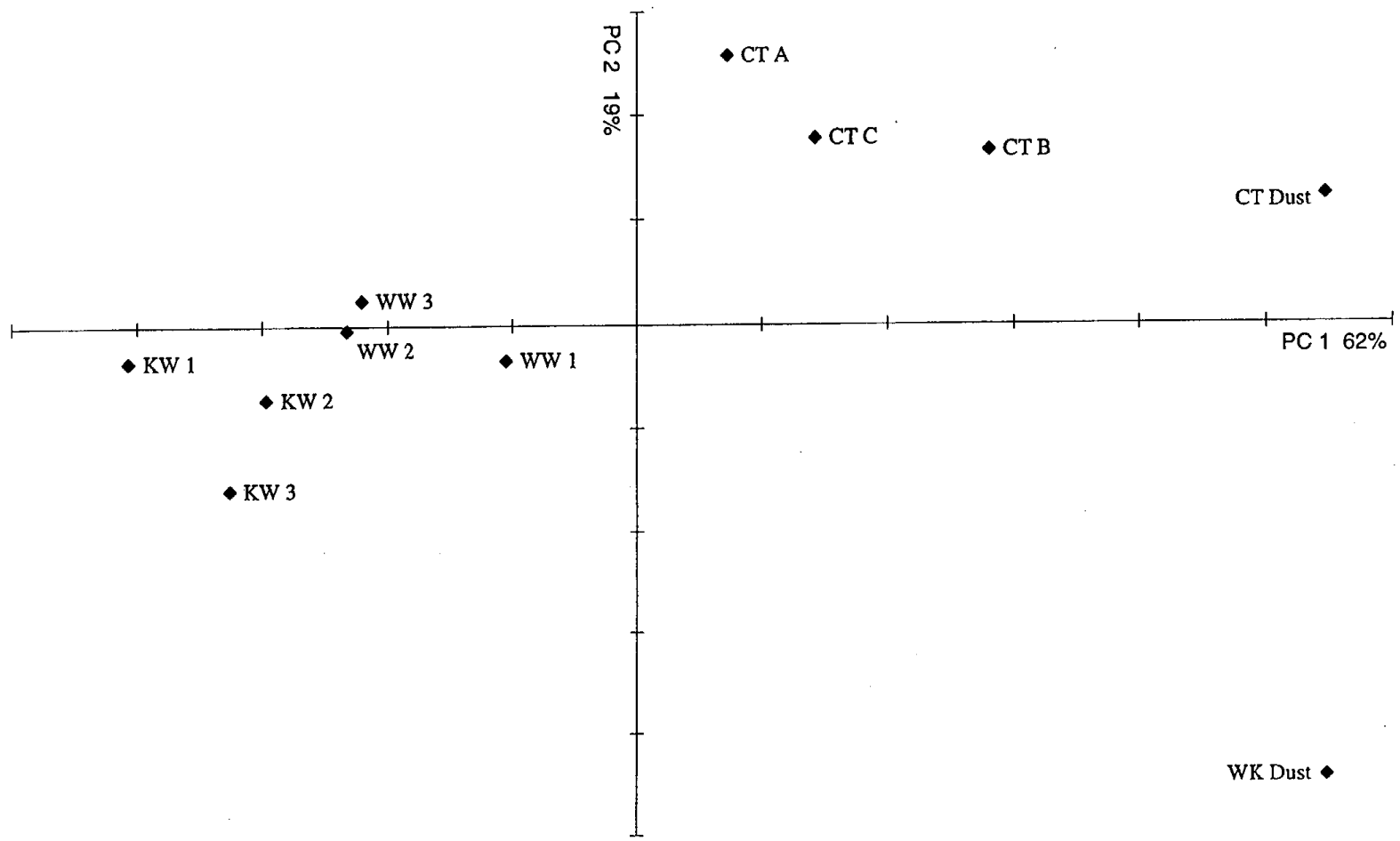

Fig. 3-16. Principal component analysis plot of PLFA fingerprints (removing those PLFAs associated with plant material) of paired dust and soil samples from: i) the Campbell tract (CT) near Davis, CA, and ii) Westhaven soil (WW), Kimberlina soil (KW), and a composite dust sample (WK) from Stoneland, CA. The first and second components represent 62 and 19%, respectively, of the variance in the data. In this plot, the differences between the Stoneland soils and dust samples were not as great as when the plant associated PLFAs were included in the analysis (e.g., Fig. 3-14).

importance of these particular PLFAs in the Campbell tract dust samples. One major difference between the two sites is that the Stone Land site is under active cultivation whereas the Campbell tract has been fallow for several years.

3.6 Analysis of PLFA data by pattern recognition and neural network methods

PLFA analysis generates complex compositional data sets that can be analyzed in a variety of ways. In the past we have used ordination methods to describe the distribution of soil PLFA profiles across two-dimensional PCA (principal component analysis) plots. Although ordination methods provide qualitative information on relationships among different soils, dust apportionment studies require quantitative measures that can be statistically tested. In light of these requirements, we enlisted the help of Dr. Phil Hopke, a professor of chemometrics at Clarkson University, to identify appropriate statistical analysis methods. We gave Dr. Hopke a data set consisting of compositional percentages of 26 PLFAs from 245 soil samples representing 13 different crop types or land uses. Hopke and associates compared three different pattern recognition methods (SIMCA, Kohonen, and ART-2a) for testing the strength of these differences (Table 3-2). To enhance statistical significance, Hopke limited the SIMCA analysis to samples representing the seven crop types which had at least four data points (232 samples). For the Kohonen and ART-2a analyses, he entered data from all 13 crop types (245 samples).

Results from Hopke's analyses indicated that over 90% of the data fell into separable classes based on crop type at the 95% confidence level. All three methods identified rice soils as being distinct from all other soils. SIMCA and ART-2a, but not Kohonen analysis, also recognized construction site samples as distinct. Although most other samples fell within classes comprising their respective crop types (e.g., tomato, cotton, almond, rotation), approximately 5% of the samples fell into other classes, creating classes with mixed composition at the 95% confidence level (Table 3-2). These results thus provide a quantitative means to evaluate relationships among soil PLFA profiles, and we will evaluate them further to see how other factors (i.e., soil type, farm management, geographic location) influenced the class distributions. Each of these three statistical analysis methods used by Hopke could be validly used to classify samples as a way of narrowing down potential single sources of dust. Kohonen and ART-2a, but not SIMCA, could also be applied to the problem of apportioning multiple sources of fugitive dust.

Hopke and associates also applied fuzzy ARTMAP and back progagation neural network techniques to analyze the same PLFA data set. These latter two methods would be suitable for both sample classification and source apportionment purposes. Both methods could clearly discriminate most soil samples based on crop type. Rice and tomato soil samples, which had the largest number of samples, were well separated from the other crop types. Crops represented by very few soil samples (<5) could not be as clearly discriminated. A manuscript describing this study by Song et al. has been submitted to the journal Chemometrics and Intelligent Laboratory Systems.

Table 3-2. Comparison of advanced multivariate analysis methods for classifying Central Valley soil samples

	SIMCA (Modeling)	SIMCA (Prediction)	Kohonen	ART-2a
Samples analyzed	232	232	245	245
No. of "crop" types among samples	7*	7*	13**	13**
No. of classes identified	7	7	15	34
Classes having 1 crop type (pure)	6 (86%)	3 (43%)	11 (73%)	29 (85%)
Classes having 2 or more crop types (mixed)	1 (14%)	4 (57%)	4 (36%)	5 (15%)
No. of samples misclassified or not classified	1	10	12	11
Percentage of samples misclassified or not classified	0.4%	4.3	4.9%	4%
No. of crop types falling into 1 pure class	6 (rice, tomato, fallow, construction, almond, rotation)	2 (rice, fallow)	4 (rice, road, compost, lake)	2 (road, lake)
No. of crop types falling into 2 or more pure classes	0	0	0	4 (rice, construction, compost, pine)
No. of crop types falling into 1 mixed class	0	1 (construction)	0	0
No. of crop types falling into 2 or more classes	1 (cotton)	4 (tomato, cotton, almond, rotation)	9 (tomato, cotton, almond, rotation, fallow, walnut, fig, pine, construction)	7 (tomato, cotton, almond, rotation, fallow, walnut, fig)

* SIMCA analysis used 232 of the 245 total samples (limited to crop types with 4 or more samples). The 7 crop types were: rice (112), tomatoes (70), fallow (4), construction (4), cotton (18), almond (11), and rotation (13). Number of samples in each crop type are in parentheses).

** Kohonen and ART-2a analyses used all 245 samples from 13 different crop types: rice (112), tomato (70), fallow (4), construction (4), pine (2), lake (2), walnut (3), cotton (18), almond (11), road (2), fig (2), rotation (13), and compost (2).

3.7. Conclusions for PLFA analyses

Redundancy analysis of PLFA fingerprints from SAFS soils showed that microbial communities in organic and conventional systems were significantly different throughout the season from April to July. On ordination plots, PLFA fingerprints from the low input usually fell between organic and conventional systems. The relative importance of environmental variables in governing the composition of microbial communities could be ranked in the order: soil type > time > specific farming operation (e.g., cover crop incorporation or sidedressing with mineral fertilizer) > management system > spatial variation in the field. Although differences could be discerned among management, season, crop, etc. within the SAFS soils, these differences were much smaller than those between the SAFS soils and cotton, almond, fig and walnut soils collected from the San Joaquin valley (the PM-10 set).

4.0 COMPARISON OF PLFA AND SFAME

Side-by-side comparisons of SFAME and PLFA analyses were made on the PM 10 soil set. Since SFAME extracts the majority of fatty acids present in a sample and PLFA extracts a subset of the total, the two methods extract different sets, and amounts, of fatty acids. We also evaluated the feasibility of using smaller samples sizes for SFAME analysis, since dust samples collected for PM_{10} analysis typically range from 2-3 micrograms up to 100 micrograms. A 100-microgram sample of a high-organic matter soil produced 20 detectable peaks in the SFAME fingerprint, which may provide sufficient information for differentiating samples. A 100-microgram sample of a low-organic matter soil produced 10 detectable peaks, and these results were not reproducible. Larger sample sizes for low-organic-matter soils would be needed to produce more information from SFAME analysis. We used two sample sizes for SFAME extractions from bulk dust (100 micrograms and 100 milligrams). We found that 100 micrograms of dust can generate enough detectable peaks for sample comparisons if we increase detection sensitivity with a splitless sample injection method. We also found that the 100-milligram samples of dust were too large for SFAME analysis, because the gas chromatography column became overloaded and caused the detector to become nonlinear.

We analyzed both SFAME and PLFA analyses using principal components to see if the two procedures give similar results (Figs. 4-1 and 4-2). Both plots showed a clear separation of the coarse almond soils and the fine cotton soils. The plots differed in that PLFA fingerprints of field replicates clustered together more tightly based on texture, while SFAME profiles clustered better based on crop type. In addition, the SFAME method was able to cluster the fig soils. This difference appears to be due to the fact that the SFAME method extracts lipid from both living and dead organisms as well as plant material, which yields a different, but similar, set of fatty acids. Within this data set it

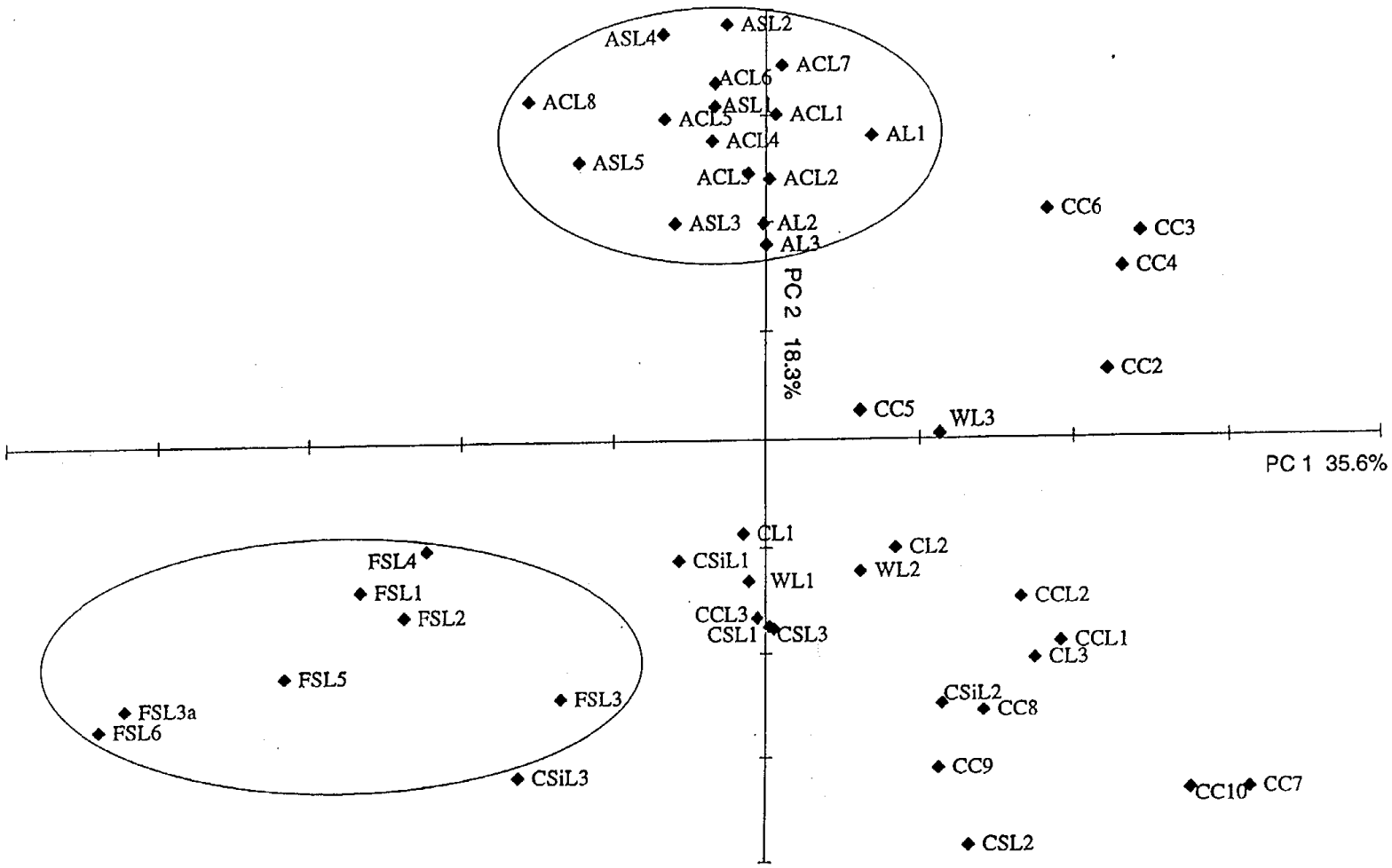

Fig. 4-1. Principal component analysis plot of SFAME fingerprints of PM-10 soils. The SFAME method was able to separate the fig soils from cotton and almond soils. When compared to the PLFA method (Fig. 3-10), SFAME produced a tighter cluster of almond soils, but the almond soils were not as clearly separated based on texture.

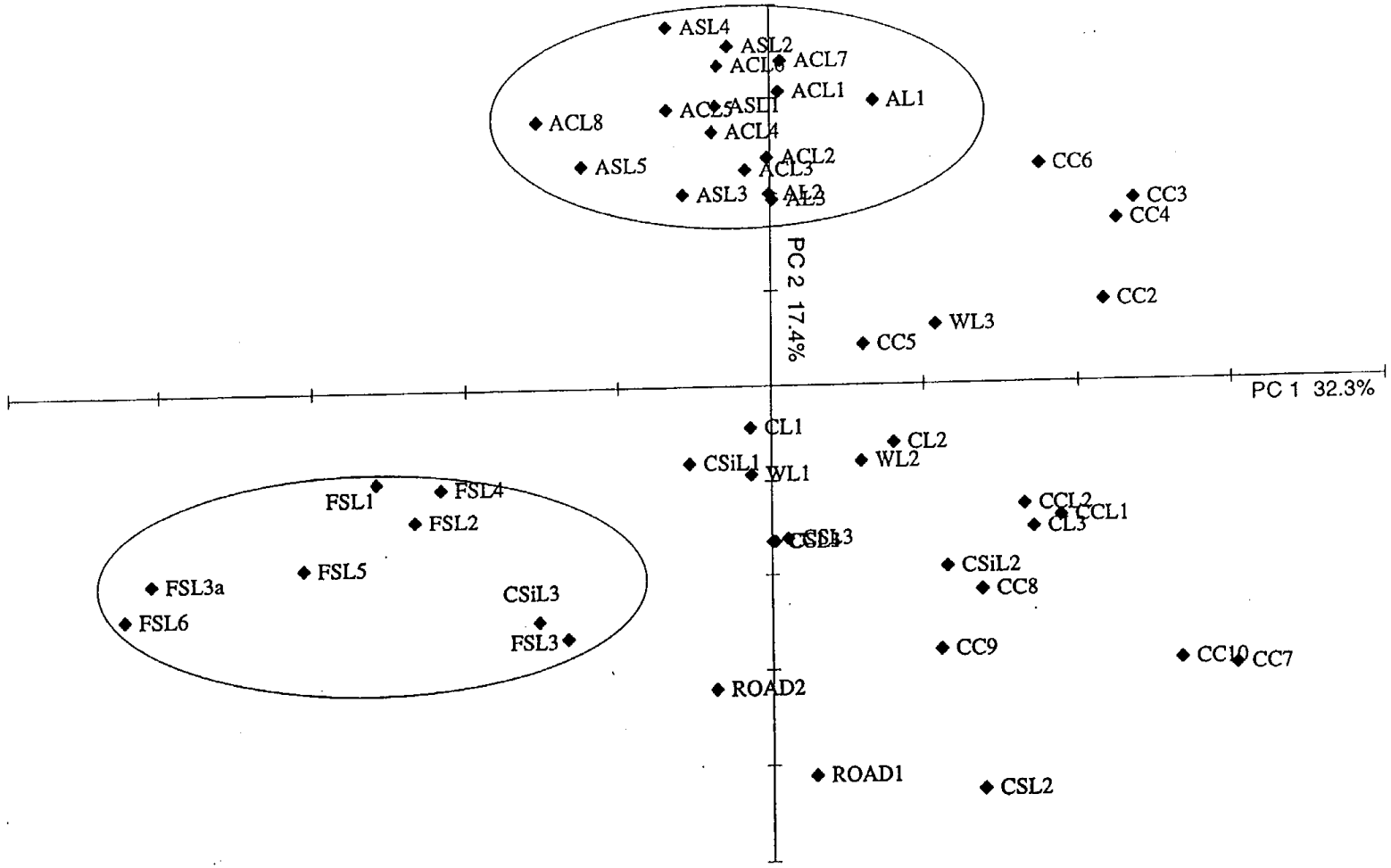

Fig. 4-2. Principal component analysis plot of SFAME fingerprints of PM-10 soils including samples collected from adjacent road. The SFAME method was less effected by the addition of the road samples than PLFA (Fig. 3-10 and 3-11).

appears that the SFAME method is capable of classifying samples based primarily on the crop and the PLFA method is more sensitive to texture.

5.0 DNA RESULTS AND DISCUSSION

5.1 DNA yields from soils and dusts.

DNA yields purified from cotton soils of varying textures ranged from 0.5 to 1.8 micrograms per gram of dry soil. DNA yields varied considerably among replicate samples of the same soil (coefficients of variation as high as 40%). This high variability appears to be due to differences in shearing with subsequent differences in losses of sheared DNA during gel purification. We did not obtain good correlation between PLFA and DNA yields from the same soil samples, even though we had expected that soils with higher PLFA yields would also have higher DNA yields.

PLFA and DNA yields from the same PM10 soil samples did not correlate well. For four of the soils, we obtained approximately the same amount of purified DNA (1 microgram per gram of dry soil), while their mean PLFA yields ranged from 100 to 600 nanograms per gram of dry soil. The poor correlation between DNA and PLFA yields is probably due to differences in the two extraction methods. The PLFA method is a harsher chemical extraction, while the DNA method is designed to keep DNA from being degraded and lost during extraction and purification. Despite the fact that our DNA extraction procedure may not have high efficiency in recovering DNA from all soils, the yields are more than adequate for producing fingerprints.

5.2 RAPD (Randomly Amplified Polymorphic DNA) analysis.

Problems were initially encountered in standardizing RAPD analysis, because we obtained different results with the same DNA sample when PCR was done on two different thermal cyclers. We purchased our own thermal cycler (Perkin-Elmer GeneAmp 2400) so that we could run PCR on the same machine thereafter. Other problems were due to variability in the amount of DNA used in the PCR reaction,. We addressed the latter problem by purchasing an adapter and microcuvets to hold 100-microliter sample volumes to measure DNA absorbance values in the UV-vis spectrophotometer. A large proportion (70-80%) of the RAPD runs were unsuccessful (i.e., no bands were obtained at all or smearing obfuscated band patterns). In the successful RAPD runs, we could see that there were distinct differences in RAPD patterns for Rindge, Forbes, Yolo, and Tinker soils, as well as some consistency in patterns obtained from the same soil or management treatment.

In a quality assurance evaluation of fingerprint consistency, we compared 20 groups of RAPDs from a data set using soils from replicated agricultural plots. We were particularly interested in comparing fingerprints produced on different days from the same extract that was used as the positive control. The strongest evidence of inconsistency in RAPD fingerprints was the difference in the number of bands from the same extract on different days. The overall pattern of the bands (i.e., positions relative to each other) was usually quite consistent, such that bands in the simplest fingerprints were also seen at the same locations in the more complex

fingerprints.. Fingerprint variability is a commonly observed problem with this method (e.g., for typing DNA from individual species of organisms.) Thus, RAPD fingerprinting may be inherently too variable to be used for fingerprinting community DNA.

5.3 PCR amplification of taxonomic genes.

Since random-primed PCR gave variable results, we chose to use group-specific primers that bind to ribosomal RNA genes of specific groups of organisms. The products of these PCR reactions, when separated by gradient gel electrophoresis, also produced banding patterns that have the advantage of providing descriptive information about the organisms in the soil that gave rise to the retrieved gene sequences. As expected, initial TGGE band patterns from the all-bacteria PCR appeared to be much more complex than patterns from nitrifier PCR. In the latter part of this project, we concentrated efforts on obtaining clear, interpretable TGGE patterns from all-bacterial PCR. However, after running a total of 30 TGGE gels, we have yet to achieve effective separation of PCR products. We have also observed what are apparently PCR artifacts with the all-bacterial primers. One of the difficulties in obtaining interpretable band patterns is the large number of experimental conditions that need to be optimized. We have used polyacrylamide concentrations ranging from 4.5 to 7%, urea concentrations ranging from 7 to 9 M, formamide concentrations from 0 to 20%, and various temperature gradients, electrophoresis buffers, and run times.

5.4 DNA based approaches show promise for characterization of dust sources and possibly dust.

The advantage of these methods lie in their low detection limits and ability to confirm fatty acid results with an independent method. These approaches require three steps: (1) extracting DNA from soil or dust; (2) using PCR to copy gene fragments from the extracted DNA; and (3) separating gene fragments with gel electrophoresis to produce DNA fingerprints. We have found that the same DNA extraction and PCR procedures must be consistently applied to all samples before fingerprints from different soils and dusts can be compared. By using the same DNA extraction method on approximately 50 different Central Valley soils, we obtained DNA yields ranging from 200 nanograms to 4 micrograms per gram of dry soil. As we expected, DNA yields per gram of bulk dust were approximately tenfold higher than yields from their corresponding surface soils, because dust tends to be enriched in organic matter. Although all-bacterial TGGE fingerprints of DNA from bulk dust and corresponding surface soils appeared similar, the band patterns were too broad and poorly separated to constitute interpretable fingerprints. We must optimize our PCR and gel electrophoresis procedures before we can obtain clearly defined fingerprints and confirm the similarities between soil and dust samples.

6.0 FUTURE WORK

6.1 Three research modules.

6.1.1 Adaptation of fingerprinting methods for fugitive dust monitoring.

The next step toward applying fingerprinting methods to PM10 is to analyze samples representative of fugitive dust that have been airborne long enough to be

collected on filters. Since a key concern is whether filters can deliver sufficient material to generate an informative fingerprint, the first objective of continued research should be to determine minimum sample sizes for different fingerprinting approaches. Minimum sample sizes will depend on the organic matter content of dust sample, and they should be determined as ranges by analyzing different dusts having low and high organic matter contents.

Minimum sample sizes also depend on the type of fingerprinting (Table 6-1). As a rule, PLFA analysis requires larger sample sizes than SFAME, while DNA analysis theoretically requires even less sample because of PCR's ability to exponentially amplify DNA. For each type of fingerprinting, minimum sample sizes would also depend on the method of analysis. For PLFA and SFAME, minimum sample sizes for GC-MS and GC-MS-MS analyses would be at least tenfold to a hundredfold lower than minimum sample sizes for GC alone (Table 6-2). We are determining whether we can get more information from small samples by increasing the peak detection sensitivity of the gas chromatograph. Preliminary tests with diluted standard mixtures indicate that we may increase sensitivity 10 to 20 fold by changing the way we inject samples into the chromatograph (i.e., instead of a split injection, use either a splitless or pulsed splitless injection.) For DNA fingerprinting, PCR analysis of predominant microorganisms would require lower sample sizes than PCR analysis of less numerous organisms.

The following discussion describes experimental objectives to be accomplished in making PLFA fingerprinting technology applicable to field monitoring of fugitive dust.

(1) Determine minimum sample sizes for low- vs. high-organic-matter dusts.

Organic matter content of dusts collected in California's Central Valley ranges from high levels for feedlot dusts to one percent for fallow agricultural dusts. The amount of PM10 typically found on 25-mm collection filters ranges from 50 to 800 micrograms. Two approaches could be used to determine minimum sample sizes for PLFA. The first approach would be to analyze progressively fewer filters or smaller pieces of filters containing PM10. The most practical filter for our purposes appears to be the 25-mm Teflon filter used by the CNL Air Quality Group in IMPROVE samplers (Interagency Monitoring for Protection of Visual Environments. This type of sampler has a medium flow rate (16.7L per min for the PM10 module and 23.2L per min for the PM2.5 module) and uses EPA-certified inlets (Sierra-Anderson. IMPROVE samplers can be fitted with 25-mm or 47-mm-diameter Teflon filters, so that the larger filter size is an option if that becomes necessary. A second approach would be to analyze known weights of suspended dust collected in the resuspension chamber at UCD's Crocker Nuclear Laboratory. It may also be helpful to size-fractionate bulk dust from the field to evaluate the upper limits of sample size, beyond which additional information is not as useful.

TABLE 6-1. Estimates of minimum sample sizes needed for fingerprinting methods.

Method	Soil (2% o.m.)	Total particulate dustfall (10% o.m.)	PM10 dust (10% o.m.)	PM10 dust (40% o.m.)
PLFA-GC	8 g	1 g	1 g	250 mg
PLFA-GC-MS	20 mg	5 mg	5 mg	1.5 mg
SFAME-GC	500 mg	100 mg	100 mg	25 mg
SFAME-GC-MS	2 mg	400 µg	400 µg	100 µg
DNA-PCR	1 mg	200 µg	200 µg	50 µg

Table 6-2. Typical ranges of sample weights collected on PM10 filters.

Type of filter and sampler	Total surface area of filter (cm^2)	Effective surface area of filter (cm^2)	Air flow rate for PM10 inlet (L per min)	Length of sampling time	Sample weights
25-mm Teflon filter with IMPROVE sampler	4.9	3.8	16.7	X	50 to 800 µg
47-mm Teflon filter with IMPROVE sampler	17.3	9.6	16.7	2.5X	125ug to 2 mg
47-mm quartz filter with high-volume filtration sampler	17.3	9.6	110	0.4X	125ug to 2 mg
8 x 11" quartz filter (20 x 25 cm) with high-volume filtration sampler	568	450 (estimated)	110	18X	6 mg to 90 mg

(2) Evaluate reproducibility of results obtained with standard sample sizes.

Once we establish the lower limits for sample sizes, we would need to evaluate reproducibility of fingerprints based on a standard sample size and storage time. First, reproducibility would need to be evaluated on multiple filters containing the same PM10 material. Point-source variability (i.e., from a single field) would also be evaluated using filters placed at different heights from the ground and at different locations and distances in the field. Temporal variability could also be studied at sites sampled daily, weekly, or seasonally. For these experiments, sampling locations and times should be selected on the basis of what is already known about PM10 (i.e., how far it travels on the average; how long it remains suspended; how it is distributed at different heights.) Half of the samples could be analyzed as unknowns to determine their relative distributions in PCA plots or in AI models constructed using data from the known samples. From discussions with Dr. Lowell Ashbaugh, a practical approach for us would be to use some of the samplers in CNL Air Quality Group's field sampling array. We propose to get samples from three modules located along one edge of the field (all are 3-meters high), which would give us 3 samples along a horizontal transect. There is also a vertical array (at 1, 3, and 9 meter heights) that could be located along the edge as well. These would be "co-located" samples to analyze spatial variability in fingerprints. "Sequential" samples to evaluate temporal variation could be taken from the same field the following week, month, etc.

The LTRAS and Campbell Tracts at the UC Davis Agronomy Fields would be particularly desirable for evaluating spatial and temporal reproducibility, because they offer opportunities to sample dusts from a variety of crops and management systems within a small area having the same soil type. We could also try to locate other university sites for this purpose (e.g., urban sampling) for additional samples. The close proximity of the UCD research fields would also facilitate sampling, because we could respond more quickly to meteorological conditions as needed.

(3) Test field-collected PM10 samples.

Having established standard sample size and field collection limitations, PM10 samples could be collected at multiple sites on the same day and analyzed for PLFA fingerprints.

(4) Evaluate fingerprinting technology for use in source apportionment.

In reality, airborne PM10 samples usually contain material from more than one source. To determine whether fingerprints can discriminate single-source PM10 from mixed PM10, known combinations of distinct dust samples (e.g., urban vs. agricultural) could be analyzed and their fingerprints compared. Again, two approaches could be used to achieve this, either with varied combinations of PM10-containing filters or with different relative amounts of resuspended dusts.

6.1.2 Development of neural network analysis of fingerprint data

Data analyses of all the above objectives would be greatly enhanced with the use of artificial intelligence (AI) methodologies. Analysis of a preliminary data set with fuzzy ARTMAP by Dr. Phil Hopke at Clarkson University has shown that fingerprints based on 26 PLFAs can distinguish nonsimilar sample points that are embedded within a field of sample points which are similar to each other (Song et al., manuscript submitted). In fact, AI programs that incorporate fuzzy logic are probably the only means by which PLFA and SFAME data can be used for source apportionment purposes.

6.1.3 Expansion of fingerprint databases.

Field application of dust monitoring will also require the capability to compare unknown fingerprints against a database of characterized fingerprints from known sources. PLFA databases for Central Valley soils and dusts could be expanded to include more soil types, crops, and land uses so that PLFA data could be used to identify potential signature peaks or sets of peaks for different soils. Beginning early next year the Air Quality Group at CNL will make available to us the soil samples they are collecting from about 50 sites in the San Joaquin Valley. These samples are being collected and distributed to other research groups involved in the ARB Technical Support Study 12.

7.0 SUMMARY AND CONCLUSIONS

Analysis of lipids extracted directly from soil is a feasible method for source identification. We have explored two methods for analyzing lipids. Phospholipid fatty acid (PLFA) analysis uses only the polar fraction of the lipid extracted directly from soil. Soil fatty acid methyl ester (SFAME) analysis quantifies all fatty acids present. Both methods can generate a fingerprint for a particular soil. With the analysis of PLFAs, we were able to differentiate many agricultural soils from numerous locations in the Central Valley of California. In addition, for a set of soils from the San Joaquin Valley, we were able to determine that crop, soil texture, and location were significant factors in determining which soils group together or which are different. An advantage of the PLFA method is the ability, in most cases, to obtain clearly isolated peaks on the chromatographs. Another strength of PLFA is the ability to relate measured data to an existing data base describing which lipids are associated with which types of organisms. A limitation of the PLFA method, as currently employed, is that it requires soil sample sizes of 1 to 8 grams and excludes some of the lipid classes from its analysis. We compared PLFA to SFAME analysis for the same set of soil samples. We found that SFAME classifies soils in a similar way to PLFA and can be performed on smaller samples (100-500 milligrams). PLFA or SFAME fingerprint data also could be used to relate bulk dust samples to their surface soils. SFAME fingerprints contain more peaks and would generate larger data sets if these peaks could be identified. A significant disadvantage of SFAME is that, because of the abundance of lipids in soil, numerous overlapping peaks are generated on the chromatographs, and individual peaks are difficult to identify with confidence. Thus, both methods have their application in the future development of technologies for source identification. DNA based approaches also show promise for characterization of dust sources and possibly dust. The advantage of DNA based methods lie in their low detection limits and ability to confirm fatty acid results with an independent method. Although all-bacterial TGGE fingerprints of DNA from

bulk dust and corresponding surface soils appeared similar, the bands were too poorly separated to constitute interpretable fingerprints. Continued attempts to optimize the PCR and gel electrophoresis procedures for soil community DNA are needed, and such work would be beneficial in the long term in providing an independent method for confirming fatty acid biomarkers by detecting the presence of microbial groups in soil and dust samples.

8.0 REFERENCES

Bossio, D.A., and K.M. Scow. 1997a. Impact of carbon and flooding on PLFA profiles and substrate utilization patterns of soil microbial communities. *Microbial Ecology* (in press).

Bossio, D.A., and K.M. Scow. 1997b. Microbial ecology in agricultural ecosystems: impacts of management changes on the microbial community in a rice production system. *California Agriculture* (in press).

Bossio, D.A., K.M. Scow, N. Gunapala, and K.J. Graham. 1997. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* (in press).

Heuer, H., and K. Smalla. 1997. Application of denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis for studying soil microbial communities. In Modern Soil Microbiology (J.D. van Elsas, J.T. Trevors, and E.M.H. Wellington, eds.), Marcel Dekker, Inc., New York, NY, pp. 353-373.

Kowalchuk, G.A., J.R. Stephen, W. de Boer, J.I. Prosser, T.M. Embley, and J.W. Woldendorp. 1997. Analysis of ammonia-oxidizing bacteria of the beta-subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Appl. Environ. Microbiol.* **63**:1489-1497.

Malik, M., J. Kain, C. Pettigrew, and A. Ogram. 1994. Purification and molecular analysis of microbial DNA from compost. J. *Microbiol. Meth.* **20**:183-196.

Mitchell, L.G., A. Bodenteich, and C.R. Merril. 1994. Use of silver staining to detect nucleic acids. In *Methods in Molecular Biology: Protocols for Gene Analysis*, vol. 31, (A.J. Harwood, ed.), Humana Press, Inc., Totowa, NJ, pp. 197-203.

Pace, N.R. D.A. Stahl, D.J. Lane, and G. Olsen. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. *Adv. Microb. Ecol.* **9**:1-55.

Sambrook, J., E.F. Fritsch, and T. Maniatis (eds.). 1989. *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Plainview, NY.

Song, X.H., P.K. Hopke, M.A. Bruns, D.A. Bossio, and K.M. Scow. A fuzzy adaptive resonance theory-supervised predictive mapping neural network applied to the classification of multivariate chemical data. *Submitted to Chemometrics and Intelligent Laboratory Systems*.

ter Braak, C.J.F., 1990. *CANOCO: A Fortran Program for Canonical Community Ordination*. Agricultural Mathematics Group, Wageningen, The Netherlands.

ter Braak, C.J.F. 1987. Ordination. *In Data Analysis in Community and Landscape Ecology*. (R.H.G. Jongmann, C.J.F. ter Braak, and O.F.R. van Tongeren), Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 91-169.

Teske, A., C. Wawer, G. Muyzer, and N.B. Ramsing. 1996. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR- amplified ribosomal DNA fragments. *Appl. Environ. Microbiol.* **62**:1405-1415.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King, and R.J. Bobbie. 1979. Determination of sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**:51-62.

Zhou, J., M.A. Bruns, and J.M. Tiedje. 1996. DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 62:316-322.

APPENDIX 9-1. List of soil samples

PM 10 SOILS																	
PM10 No.	Date	Crop	PCA code	County	Latitude	Longitude	Elevation	Array code	Sampling location	Soil texture	Soil map unit	% H_2O	% Sand	% Silt	%Clay	PLFA Nos.	DNA Nos.
241	10/17/90	Almond	ACL1	Kern	35.65	119.97	196	PF8	north	loam/clay loam	Panoche clay loam	8.39	38.53	37.08	24.39	825-827	
323		Almonds	ACL2	Kern	35.67	119.9	196	PF6	20M	Clay loam	Panoche clay loam/Kimberlina	4.80	24.76	38.98	36.26	483-485	88-90
324		Almonds	ACL3	Kern	35.67	119.9	196	PF6	30M	Clay loam	Panoche clay loam/Kimberlina	4.17	24.17	40.75	35.07	498-500	91-93
325		Almonds	ACL4	Kern	35.67	119.9	196	PF6	40M	Clay loam	Panoche clay loam/Kimberlina	4.01	31.59	37.82	30.58	828-830	
326		Almonds	ACL5	Kern	35.67	119.9	196	PF6	5S	Clay loam	Panoche clay loam/Kimberlina	4.75	22.69	40.56	36.76	584-586	94-96
327		Almonds	ACL6	Kern	35.67	119.9	196	PF6	10S	Clay loam	Panoche clay loam/Kimberlina	3.72	25.28	41.34	33.37		
330		Almonds	ACL7	Kern	35.67	119.9	196	PF6	40S	Clay loam	Panoche clay loam/Kimberlina	5.79	29.59	38.03	32.38	831-833	
331		Almonds	ACL8	Kern	35.67	119.9	196	PF6	R 1/2		Panoche clay loam/Kimberlina		32.64	36.46	30.90	834-836	
236	10/16/90	Almond	AL1	Kern	35.67	119.97	196	PF7	N. 50m	loam	Panoche clay loam	5.70	39.61	35.50	24.89	501-503	147-179
237	10/16/90	Almond	AL2	Kern	35.67	119.97	196	PF7	middle	loam	Panoche clay loam	5.90	41.79	35.04	23.17	504-506	150-152
238	10/16/90	Almond	AL3	Kern	35.67	119.97	196	PF7	S. 50m	loam	Panoche clay loam	5.82	40.23	34.81	24.96	588-590	153-155
276		Almonds	ASL1	Kern	35.5	119.17	105	PF2	5S	sandy loam	Driver coarse sandy loam	1.44	62.29	22.14	15.57	507-509	135-137
277		Almonds	ASL2	Kern	35.5	119.17	105	PF2	11N	sandy loam	Driver coarse sandy loam	1.63	60.16	23.78	16.06	510-512	138-140
286		Almonds	ASL3	Kern	35.5	119.17	105	PF2	29N	sandy loam	Driver coarse sandy loam	1.41	63.73	23.32	12.95	591-593	141-143
294		Almonds	ASL4	Kern	35.52	119.17	105	PF3	35N	sandy loam	Lewkalb/Wasco sandy loam	1.07	65.42	24.23	10.35	393-395	22-24
296		Almonds	ASL5	Kern	35.52	119.17	105	PF3	47M	sandy loam	Lewkalb/Wasco sandy loam	0.71	65.11	24.96	9.93	533-535	156-158
433		Cotton	CC4	Kern	35.18	119.33	90	BV2	50	clay	Copus silt clay	5.86	4.01	38.18	57.80	568-570	113-115
438		Cotton	CC6	Kern	35.18	119.33	90	BV2	250	clay	Copus silt clay	7.60	4.18	37.65	58.16	549-551	116-118
422		Cotton	CC8	Kings	36.07	119.8	62	CR1	350	clay	Tulare clay	5.92	1.34	37.06	61.60	879-881	
441	11/8/90	Cotton	CC10	Kings	36.12	119.85	61	NB1	E3 (?)	clay	Tulare clay		0.70	32.79	66.51	844-846	
442	11/8/90	Cotton	CC7	Kings	36.12	119.85	61	NB1	W4 (?)	clay	Tulare clay		0.72	34.78	64.50	847-849	
233	10/20/90	Cotton	CCL1	Fresno	36.25	120.05	84	BR3	row 10 N.	clay loam	Lethent/Westhaven clay loam	8.07	32.95	35.23	31.82	378-380	19-21, 100-102
234	10/20/90	Cotton	CCL2	Fresno	36.25	120.05	84	BR3	row 50 N.	clay loam	Lethent/Westhaven clay loam	8.59	32.24	35.82	31.94		103-105
235	10/20/90	Cotton	CCL3	Fresno	36.25	120.05	84	BR3	row 125 N.	clay loam	Lethent/Westhaven clay loam	12.22	27.03	42.74	30.24	572-574	106-108
405	10/2/90	Cotton	CL1	Kern	35.17	118.93	106		10m	loam	Oldriver loam	5.11	44.72	42.88	12.40	384-386	
406	10/2/90	Cotton	CL2	Kern	35.17	118.93	106		100m	loam	Oldriver loam	4.90	49.32	38.78	11.90	492-494	128-130
403	10/2/90	Cotton	CL3	Kern	35.17	118.93	106		100m	loam	Oldriver loam	8.70	49.55	40.59	9.85	578-580	125-127
367	10/9/90	Cotton	CC1	Kern	35.18	119.28	90	BV1	50M	clay	Copus silt clay/Buttonwillow clay	7.43	6.70	37.97	55.34	372-374	14-15
437		Cotton	CC5	Kern	35.18	119.33	90	BV2	150	clay/silt clay	Copus silt clay	7.29	4.26	39.93	55.81	375-377	16-18
368	10/9/90	Cotton	CC2	Kern	35.18	119.28	90	BV1	100M	clay/silt clay	Copus silt clay/Buttonwillow clay	7.71	6.18	39.69	54.13	486-488	97-99
372	10/9/90	Cotton	CC3	Kern	2.14	119.28	90	BV1	100S	clay	Copus silt clay/Buttonwillow clay	6.11	5.94	38.89	55.18	565-567	109-112
425		Cotton	CC9	Kings	36.07	119.8	62	CR1	350	clay/silt clay	Tulare clay	6.79	1.70	39.10	59.20	882-884	
395	10/7/90	Cotton	CSiL1	Kern	35.17	118.93	106	KL3	150m	silt loam	Weedpatch clay loam/Lokern clay	5.76	26.31	51.90	21.79	575-577	122-124
398	10/7/90	Cotton	CSiL2	Kern	35.17	118.93	106	KL3	50m	silt loam	Weedpatch clay loam/Lokern clay	6.95	26.90	51.74	21.36	381-383	
400	10/7/90	Cotton	CSiL3	Kern	35.17	118.93	106	KL3	250m	silt loam	Weedpatch clay loam/Lokern clay	4.59	27.18	51.24	21.58	489-491	119-121
407	10/2/90	Cotton	CSL1	Kern	35.17	118.93	106		200m	sandy loam	Oldriver loam	2.29	63.81	24.12	12.07	417-419	131-133
402	10/2/90	Cotton	CSL2	Kern	35.17	118.93	106		10m	sandy loam	Oldriver loam	3.22	67.39	20.80	11.81	387-389	
404	10/2/90	Cotton	CSL3	Kern	35.17	118.93	106		200m	sandy loam	Oldriver loam	2.71	66.77	30.10	3.13	581-583	144-146
254	9/28/90	Figs	FSL1	Merced	37.12	120.27	42	DE2	row 2 E. column	sandy loam	Hanford/Tujunga sandy loam	2.32	66.62	24.75	8.63	552-554	174-176
258	9/28/90	Figs	FSL2	Merced	37.12	120.27	42	DE2	row 12 E. column	sandy loam	Hanford/Tujunga sandy loam	2.73	67.38	24.35	8.27	556-558	177-179
268	9/28/90	Figs	FSL3	Merced	37.12	120.27	42	DE2	row 37 E. column	sandy loam	Hanford/Tujunga sandy loam	2.28	66.60	25.79	7.62	838-840	
269	9/28/90	Figs	FSL3a	Merced	37.12	120.27	42	DE2	row 37 W. column	sandy loam	Hanford/Tujunga sandy loam	1.81	66.67	25.64	7.69		
255	9/28/90	Figs	FSL4	Merced	37.12	120.27	42	DE2	row 2 W. column	sandy loam	Hanford/Tujunga sandy loam	1.81	75.99	18.26	5.75	841-843	
257	9/28/90	Figs	FSL5	Merced	37.12	120.27	42	DE2	row 7 W. column	sandy loam	Hanford/Tujunga sandy loam	1.59	75.31	18.49	6.20	850-852	
261	9/28/90	Figs	FSL6	Merced	37.12	120.27	42	DE2	row 17 W. column	loamy sand/sl	Hanford/Tujunga sandy loam	1.05	75.88	18.01	6.11	876-878	
249	9/28/90	Road	ROAD1	Merced					east	loamy sand	Hanford/Tujunga sandy loam	0.84	84.21	11.93	3.86	559-561	168-170
251	9/28/90	Road	ROAD2	Merced					M(middle)	loamy sand	Hanford/Tujunga sandy loam	0.78	84.25	12.07	3.68		171-173
200	10/22/90	Walnuts	WL1	Kings	36.38	119.65	62	WH1	W.row1	loam	Nord fine sandy loam	3.09	49.91	38.96	11.13	536-538	159-161
202	10/22/90	Walnuts	WL2	Kings	36.38	119.65	62	WH1	WN.3rd row IN1	loam	Nord fine sandy loam	6.48	50.40	38.79	10.80	539-541	162-164
208	10/21/90	Walnuts/grass	WL3	Kings	36.38	119.67	76	FU1	W. 20th row	loam	Nord fine sandy loam	15.73	51.82	39.24	8.94	543-545	165-167

APPENDIX 9-1. List of soil samples

MISC. SOILS								
Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
Maxwell Rice Straw Study	plot 1J3	1/3/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 3	1/3/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 7	1/3/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 8	1/3/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 9	1/3/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 11	1/3/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 14	1/3/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 15	1/3/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 19	1/3/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 20	1/3/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 22	1/3/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 24	1/3/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 26	1/3/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 28	1/3/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 31	1/3/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 32	1/3/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 1F2	2/2/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 3	2/2/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 7	2/2/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 8	2/2/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 9	2/2/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 11	2/2/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 14	2/2/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 15	2/2/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 19	2/2/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 20	2/2/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 22	2/2/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 24	2/2/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 26	2/2/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 28	2/2/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 31	2/2/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 32	2/2/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 1M7	3/7/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 3	3/7/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 1

APPENDIX 9-1. List of soil samples

Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
Maxwell Rice Straw Study	plot 7	3/7/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 8	3/7/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 9	3/7/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 11	3/7/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 14	3/7/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 15	3/7/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 19	3/7/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 20	3/7/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 22	3/7/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 24	3/7/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 26	3/7/94	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 28	3/7/94	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 31	3/7/94	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 32	3/7/94	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 1F27	2/27/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 2	2/27/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 3	2/27/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 4	2/27/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 5	2/27/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 6	2/27/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 7	2/27/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 8	2/27/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 9	2/27/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 10	2/27/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 11	2/27/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 12	2/27/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 13	2/27/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 14	2/27/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 15	2/27/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 16	2/27/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 17	2/27/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 18	2/27/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 19	2/27/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 20	2/27/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 21	2/27/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 22	2/27/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 3

APPENDIX 9-1. List of soil samples

Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
Maxwell Rice Straw Study	plot 23	2/27/95	Rice	remobed, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 24	2/27/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 25	2/27/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 26	2/27/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 27	2/27/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 28	2/27/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 29	2/27/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 30	2/27/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 31	2/27/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 32	2/27/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 1A10	4/10/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 2	4/10/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 3	4/10/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 4	4/10/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 5	4/10/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 6	4/10/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 7	4/10/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 8	4/10/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 1
Maxwell Rice Straw Study	plot 9	4/10/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 10	4/10/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 11	4/10/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 12	4/10/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 13	4/10/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 14	4/10/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 15	4/10/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 16	4/10/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 2
Maxwell Rice Straw Study	plot 17	4/10/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 18	4/10/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 19	4/10/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 20	4/10/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 21	4/10/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 22	4/10/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 23	4/10/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 24	4/10/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 3
Maxwell Rice Straw Study	plot 25	4/10/95	Rice	removed, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 26	4/10/95	Rice	incorporated, not flooded	Clay	Willows Clay	Colusa	Field 4

APPENDIX 9-1. List of soil samples

Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
Maxwell Rice Straw Study	plot 27	4/10/95	Rice	rolled, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 28	4/10/95	Rice	burned, not flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 29	4/10/95	Rice	removed, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 30	4/10/95	Rice	rolled, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 31	4/10/95	Rice	burned, winter flooded	Clay	Willows Clay	Colusa	Field 4
Maxwell Rice Straw Study	plot 32	4/10/95	Rice	incorporated, winter flooded	Clay	Willows Clay	Colusa	Field 4

APPENDIX 9-1. List of soil samples

MISC. SOILS Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
LTRAS	1-5B		Tomato	conventional	Silt Loam	Yolo silt loam	Yolo	conventional
LTRAS	7-9B		Tomato	conventional	Silt Loam	Yolo silt loam	Yolo	conventional
LTRAS	8-8A		Tomato	organic	Silt Loam	Yolo silt loam	Yolo	organic
LTRAS	1-2A		Tomato	organic	Silt Loam	Yolo silt loam	Yolo	organic
U.C.D. Student Farm	YOLO 1	8/1/95	Fallow	fallow	Silt Loam	Yolo silt loam	Yolo	Fallow agricultural
U.C.D. Student Farm	YOLO 2	8/1/95	Fallow	fallow	Silt Loam	Yolo silt loam	Yolo	Fallow agricultural
Sacramento Delta (Rio Vista)	Rindge1		Fallow	fallow	Peat/Muck	Rindge	Sac'to	Organic Matter Content >40%
Sacramento Delta (Rio Vista)	Rindge2		Fallow	fallow	Peat/Muck	Rindge	Sac'to	Organic Matter Content >40%
Mace Ranch Construction (Davis)	mace Grass		Construction	construction			Yolo	grassy area
Mace Ranch Construction (Davis)	Mace Unplowed		Construction	construction			Yolo	unplowed area
Mace Ranch Construction (Davis)	Mace Construction		Construction	construction			Yolo	topsoil removed
Mace Ranch Construction (Davis)	Mace Plowed		Construction	construction			Yolo	plowed area
Auburn Vicinity	F10-1	4/1/94	Pine forest			Forbes	Placer	pine forest
Auburn Vicinity	F20-1	4/1/94	Pine forest			Forbes	Placer	pine forest
Clear Lake Sediment	SED'MNT H0	5/1/97	Lake sediment		lake sediment		Lake	lake sediment
Clear Lake Sediment	SED'MNT H10	5/1/97	Lake sediment		lake sediment		Lake	lake sediment
BIFS	B1	1/23/97	Spinich	Organic			Fresno	Farm A, manure
BIFS	B2	1/23/97	Spinich	Organic			Fresno	Farm A, manure
BIFS	B3	1/23/97	Spinich	Organic			Fresno	Farm A, manure
BIFS	B4	1/23/97	Fallow	Conventional			Fresno	Farm A, conv. rotat.
BIFS	B5	1/23/97	Fallow	Conventional			Fresno	Farm A, conv. rotat.
BIFS	B6	1/23/97	Fallow	Conventional			Fresno	Farm B, conv. rotat.
BIFS	B7	1/23/97	Fallow	Conventional			Fresno	Farm B, conv. rotat.
BIFS	B8	1/23/97	Barley Cover	Organic			Fresno	Farm C, cover crop
BIFS	B9	1/23/97	Fallow	Conventional			Fresno	Farm C, fallow conv.
BIFS	B10	1/23/97	Barley-Vetch	Organic			Fresno	Farm D, cover crop
BIFS	B11	1/23/97	Fallow	Conventional			Fresno	Farm D, fallow conv.
COMPOST	Yard		Compost				Yolo	Yard compost
COMPOST	Kitchen		Compost				Yolo	Kitchen compost
Carpenteria Organic Farm (FABER)	F1		Rotation	Organic			Ventura	Cover Crop w/ Grazing
Carpenteria Organic Farm (FABER)	F2		Rotation	Organic			Ventura	Cover Crop Only
Carpenteria Organic Farm (FABER)	F3		Rotation	Organic			Ventura	Cover Crop w/ Compost
Carpenteria Organic Farm (FABER)	F4		Rotation	Organic			Ventura	Cover Crop w/ Compost
Carpenteria Organic Farm (FABER)	F5		Rotation	Organic			Ventura	Cover Crop Only
Carpenteria Organic Farm (FABER)	F6		Rotation	Organic			Ventura	Cover Crop w/ Grazing

APPENDIX 9-1. List of soil samples

Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
Carpenteria Organic Farm (FABER)	F7		Rotation	Organic			Ventura	Cover Crop w/ Grazing
Carpenteria Organic Farm (FABER)	F8		Rotation	Organic			Ventura	Cover Crop Only
Carpenteria Organic Farm (FABER)	F9		Rotation	Organic			Ventura	Cover Crop w/ Compost
Nematode Farming Experiment (NFX)	oct(+S+)1	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	oct(+S+)2	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	oct(---)1	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	oct(---)2	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	oct(-w+)1	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	oct(-w+)2	Oct-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(+S+)1	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(+S+)2	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(---)1	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(---)2	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(-w+)1	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	nov(-w+)2	Nov-95	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(+S+)1	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(+S+)2	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(---)1	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(---)2	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(-w+)1	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	mar(-w+)2	Mar-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-s+)1	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-s+)2	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-s+)1	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-s+)2	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-w-)1	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-w-)2	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-w-)3	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
Nematode Farming Experiment (NFX)	apr(-w-)4	Apr-96	Rotation		silt loam	Yolo silt loam	Yolo	
WET-UP	0DC1		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	0DC2		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	0DO1		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	0DO2		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	0SC1		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	0SC2		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	0SO1		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth

APPENDIX 9-1. List of soil samples

SAFS SOILS Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
4/4/95TOM-O	4/8/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
4/4/95TOM-L	4/8/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
4/4/95TOM-C	4/8/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
4/18/95TOM-O	4/18/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
4/18/95TOM-L	4/18/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
4/18/95TOM-C	4/18/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/9/95TOM-O	5/9/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/9/95TOM-L	5/9/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/9/95TOM-C	5/9/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/23/95TOM-O	5/23/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/23/95TOM-L	5/23/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
5/23/95TOM-C	5/23/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/3/95TOM-O	7/3/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/3/95TOM-L	7/3/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/3/95TOM-C	7/3/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/28/95TOM-O	7/28/95	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/28/95TOM-L	7/28/95	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
7/28/95TOM-C	7/28/95	Tomato	conventional	silt loam	Yolo silt loam	Yolo	Average of 4 Plots (Individual Replications are plotted in Figure #?
96TOM-O	7/8/96	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
96TOM-L	7/8/96	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
96TOM-C	7/8/96	Tomato	conventional 4 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
96TOM-H	7/8/96	Tomato	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97TOM-O	7/28/97	Tomato	organic	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97TOM-L	7/28/97	Tomato	low input	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97TOM-C	7/28/97	Tomato	conventional 4 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97TOM-H	7/28/97	Tomato	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97COR-O	7/28/97	Corn	organic	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97COR-L	7/28/97	Corn	low input	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97COR-C	7/28/97	Corn	conventional 4 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97COR-H	7/28/97	Corn	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97SAF-O	7/28/97	Safflower	organic	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97SAF-L	7/28/97	Safflower	low input	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97SAF-C	7/28/97	Safflower	conventional 4 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97SAF-H	7/28/97	Safflower	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97BEA-O	7/28/97	Bean	organic	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97BEA-L	7/28/97	Bean	low input	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97BEA-C	7/28/97	Bean	conventional 4 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97BEA-H	7/28/97	Bean	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots
97WHE-H	7/28/97	Wheat	conventional 2 year rotation	silt loam	Yolo silt loam	Yolo	Average of 3 Plots

APPENDIX 9-1. List of soil samples

Sample Set	Sample Name	Date	Crop	Mangement	Soil Texture	Soil Name	County	Notes
WET-UP	0SO2		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	1DC1		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	1DC2		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	1DO1		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	1DO2		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	1SC1		Tomato	conventional	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	1SC2		Tomato	conventional	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	1SO1		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	1SO2		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	24DC1		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	24DC2		Tomato	conventional	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	24DO1		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	24DO2		Tomato	organic	silt loam	Yolo silt loam	Yolo	5-15cm Depth
WET-UP	24SC1		Tomato	conventional	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	24SC2		Tomato	conventional	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	24SO1		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
WET-UP	24SO2		Tomato	organic	silt loam	Yolo silt loam	Yolo	0-5cm Depth
Campbell Tract	CT-A		Fallow		silt loam	Yolo silt loam	Yolo	
Campbell Tract	CT-B		Fallow		silt loam	Yolo silt loam	Yolo	
Campbell Tract	CT-C		Fallow		silt loam	Yolo silt loam	Yolo	

APPENDIX 9-2

PROTOCOL FOR PHOSOPHOLIPID FATTY ACID (PLFA) ANALYSIS OF SOIL SAMPLES

Overview.

Analysis of PLFAs provides insight into the structure and status of the soil microbial community. Phospholipids are quickly degraded upon the death of a microorganism. Fatty acids derived from phospholipids represent the potentially viable members of a community. Soil lipids are extracted directly from the sample. Phospholipids are separated from the other lipid classes for analysis by gas chromatography.

Sample precautions.

Sample contamination can be reduced by proper laboratory practices. Reagents are of the highest grade possible e.g. pesticide grade solvents. All procedures are carried out in either Teflon or glass. Rinse labware with hexane prior to use. Fatty acids from oils on the skin may appear in the analysis. Never directly handle samples or anything that will come in contact with the sample. Use nitrile gloves at all times. They are more resistant to the solvents used than latex, or most other common glove materials. Keep samples frozen (-20° C or lower) until ready for analysis. Avoid thawing and refreezing of samples.

Personal safety.

Read the material safety data sheet for all reagents used. Work in an approved fume hood and wear gloves.

Reagents and Supplies

Glacial acetic acid (for 1.0 M Acetic Acid)
Acetone
Chloroform
Hexane
Methanol
Methyl Nonadecanoate (recommend internal standard for GC)
Nano-Pure® water, or equivalent
Potassium phosphate, dibasic (for 0.05 M phosphate buffer)
Sodium hydroxide pellets (for 0.2 M KOH in MeOH)
Toluene
Silica gel solid phase extraction cartridges, 500mg, 3 ml
Disposable Pasteur pipette, 5¾ inch and 9 inch
11 mm GC vial, Teflon lined crimp top cap, 250 ml insert
Disposable Micro-Pipettor glass tubes
¼ Disposable vials with Teflon lined caps
99.9995% Pure hydrogen, helium and air for GC
99.99% Pure nitrogen for sample evaporation

Equipment and Instrumentation

35 ml Teflon centrifuge tubes
Shaker
Centrifuge
125 ml separatory funnels with Teflon stopcocks
13x100mm test tubes with Teflon lined phenolic caps
10x50mm test tubes with Teflon lined phenolic caps
Sample drying apparatus
Solid phase extraction cartridge rack
Water bath
Freezer

50-250 µl Digital Micro-Pipettor with glass capillary tubes??
10 ml x 1 ml pipette
1 ml x 0.1 ml pipette
GC vial crimper
Gas chromatograph with:
flame ionization detector
25 M x 0.20 mm I.D. x ??mm
autosampler
chromatography and peak identification software

Procedure

Use 35 ml Teflon centrifuge tubes that have been washed, dried, and rinsed with hexane.
For 8 grams, dry weight, of soil for an analysis:

$$8.0 \times (1 + \theta) = \text{Mass of moist soil to be weighed out.}$$

Bring total H2O in the initial extraction to 5 ml using PO4 buffer, while accounting for the soil's water content. The volume of water in the soil is equal to the mass of moist soil minus the 8 grams of dry soil, based on 1 gram / 1 ml. Subtract amount of water in soil from 5 ml of P buffer to determine the amount used in the centrifuge tube.
Example of Calculation: Soil "X" is at 16% soil moisture content,

$$8 \times 1.16 = 9.28 \text{ grams of moist soil and } 3.72 \text{ ml of P buffer required.}$$

Extraction

1) Add total of 5 ml of P buffer (see above, be sure to account for soil moisture content), plus 6 ml of $CHCl_3$ and 12 ml of MeOH.
2) Shake for 2 hours.
3) Centrifuge at 2500 rpm for 10 min. at 25° C.
4) Decant to separatory funnel.
5) Add 23 ml of Extractant ($CHCl_3$:MeOH:Buffer in a 1:2:0.8 ratio) to soil remaining in tube, vortex.
6) Shake for ½ hour.
7) Add 12 ml of $CHCl_3$ and 12 ml of P buffer to sep funnel (Add this while waiting for step # 6).
8) Centrifuge at 2500 rpm for 10 min. at 25° C.
9) Decant this to the same sep funnel.
10) Shake sep funnel for 2 minutes.
11) Let stand overnight for separation.
12) Clean centrifuge tubes: Fill half full with water, cap, vortex, dispose of soil in waste. Wash tubes with soap and hot water, rinse w/ hot tap 5x, DI 5x, nanopure 3x.

Next Day

1) Drain bottom layer from sep funnel into large diameter long glass test tubes .
2) Evaporate with N_2 at 30° - 32° C in water bath.

Conditioning Solid Phase Extraction cartridges (SPE), Transfer of lipids, and Fractionation

1) Use 10x50mm test tubes for SPE fraction collection.
2) Add 3 ml of $CHCl_3$ to condition column.
3) Transfer lipids with four (4X) 250µl transfers of $CHCl_3$, using digital micro-pipetor.
4) Add 5 ml of $CHCl_3$.
5) Add 10 ml of Acetone.
6) Change collection tubes.
7) Add 5 ml of MeOH, Be sure to save this fraction.
8) Evaporate with N_2 at 32° C in water bath.

Transesterification

1) Add 1 ml of 1:1 MeOH:Toluene, and 1 ml of 0.2 M KOH, to the dried sample. Vortex.
2) Heat at 37 ° for 15 min. in the water bath.
3) After heating, add 0.3ml of 1.0 M acetic acid, then 2 ml of hexane, then 2 ml of nanopure water, then cap and shake for 10 minutes on low setting.

4) Remove the upper layer to small disposable screw top vials.
5) Repeat the 2 ml of Hexane, shake for another 10 minutes.
6) Remove this upper layer and add it to the first hexane fraction.
7) Dry with N_2. (No water bath required).

Preparation for GC

1) Use small crimp seal G.C. vials with inserts.
2) Transfer (Use and save glass pipettes) with two 75 µl additions of 19:0 internal standard. The concentration of the internal standard depends on the expected concentration of fatty acids in your sample. Recommend 25ng/µl.
3) Purge with N_2 and seal.
4) Store sealed G.C. vials in the freezer until analysis.

APPENDIX 9-3

PROTOCOL FOR SOIL FATTY ACID METHYL ESTER (SFAME) ANALYSIS OF SOIL SAMPLES

Overview.
The diversity within the soil ecosystem can be used as a source of a soil's fingerprint. The information used for this fingerprint is based on the biological component of soil. Microorganisms and plant residues are intimately tied together and form the active portion of a soil's carbon pool. Direct extraction of this pool and analysis of the chemically modified compounds can be performed on samples of limited mass.

Sample precautions.
Sample contamination can be reduced by proper laboratory practices. Reagents are of the highest grade possible e.g. pesticide grade solvents. All procedures are carried out in either Teflon or glass. Rinse labware with hexane prior to use. Fatty acids from oils on the skin may appear in the analysis. Never directly handle samples or anything that will come in contact with the sample. Use nitrile gloves at all times. They are more resistant to the solvents used than latex, or most other common glove materials. Keep samples frozen (-20° C or lower) until ready for analysis. Avoid thawing and refreezing of samples.

Personal safety.
Read the material safety data sheet for all reagents used. Work in an approved fume hood and wear gloves.

Reagents and Supplies
Hexane
Methanol
Methyl-tert-buytl ether (MTBE)
Methyl Nonadecanoate (recommend internal standard for GC)
Nano-Pure® water, or equivalent
Potassium hydroxide pellets
6.0 N Hydrochloric acid
Sodium chloride
Disposable Pasteur pipette, 5¾ inch and 9 inch
11 mm GC vial, Teflon lined crimp top cap, 250 µl insert
Disposable Micro-Pipettor glass tubes
¼ oz. disposable vials with Teflon lined caps
99.9995% Pure hydrogen, helium and air for GC
99.99% Pure nitrogen for sample evaporation

Equipment and Instrumentation
50 ml Teflon centrifuge tubes
Shaker
Centrifuge
Sample drying apparatus
Water bath
Hot Plate
Freezer
50-250 µl Digital Micro-Pipettor
10 ml x 1 ml pipette
1 ml x 0.1 ml pipette
GC vial crimper
Gas chromatograph with:
- flame ionization detector
- 25 M x 0.20 mm I.D. x 0.33 µm
- autosampler
- chromatography and peak identification software

Procedure (based on 500 mg samples)
Saponification

1) Add 1.0 ml of 3.75N Alkaline methanol, tighten caps.
2) Vortex 5-10 seconds.
3) Place in 100°C water bath for 5 minutes. Ensure methanol is not boiling.
4) Remove and vortex for 5-10 seconds. Check tightness of caps.
5) Place in 100°C water bath for additional 25 minutes.
6) Remove and place in room temperature water bath.

Methylation

1) Add 2.0 ml of 3.25N Acidic methanol.
2) Cap and vortex 5-10 seconds.
3) Place in 80°C water bath for 10 minutes.
4) Remove and place in room temp water bath.

Extraction

1) Add 1.25 ml of hexane MTBE mix (1:1).
2) Gently shake for 10 minutes.
3) Centrifuge for 10 minutes at 2000 R.P.M.
4) Remove upper layer to disposable glass vial.
5) Repeat steps 1 through 4.

Transfer to GC vial

1) Evaporate sample with nitrogen.
2) Transfer with two 75 µl transfers using the internal standard.
3) Purge with nitrogen and cap.

APPENDIX 9-4

PROTOCOLS FOR DNA EXTRACTION AND PURIFICATION FROM SOIL SAMPLES

I. DNA EXTRACTION

Materials: Autoclaved Oak Ridge tubes (40-ml polypropylene screw-capped); Falcon tubes (50-mL polypropylene conical); 65C water bath; shaker incubator @ 37C; sterile stir sticks; high-speed centrifuges (eg., Sorvall RC 5C and Eppendorf microcentrifuge 5415).

Reagents: Sterile extraction buffer (100 mM sodium phosphate, pH 8; 100 mM NaEDTA; 100 mM Tris base, pH 8; 1.5 M NaCl; 1% CTAB) at a final pH of 8; proteinase K (10 mg/ml); 20% sodium dodecyl sulfate (SDS); isopropanol; mixture of chloroform:isoamyl alcohol at a ratio of 24:1; Tris-EDTA (TE) buffer; Nanopure water filtered through 0.2-micron filter; all solutions should be autoclaved prior to use to destroy nucleases that could degrade DNA.

Procedure:

1. Weigh out approximately 5 grams soil (wet weight) into Oak Ridge tube.[3] (Determine moisture content on separate subsamples, so that DNA yields can be calculated per gram dry soil.)

2. Add 13.5 ml extraction buffer and 50-microliters proteinase K solution to each tube. Shake tubes horizontally at 37C @ 200-225 rpm. This step is intended to remove soil nucleases.)

3. Add 1.5 ml 20% SDS to sample. Place tube in 65C water bath for 2 hours, mixing the contents every 15-20 min by inverting the tube. Once SDS is added, the sample should be mixed gently to avoid shearing the DNA.

4. Centrifuge the sample @ 6000 x g for 10 min at room temperature. Transfer supernatant to a 50-ml Falcon tube. Add 4.5 ml fresh extraction buffer to the pellet and resuspend soil by stirring gently with a sterile stick. Add 0.5 ml SDS after the pellet is resuspended.

5. Heat the sample again at 65C for 15 min. Then, repeat Step 4, transferring the second supernatant to the tube containing the first supernatant.

6. Heat the sample again for 15 min. Then, centrifuge the sample @ 12,000 x g and add the third supernatant to the first two.

7. Determine the volume of pooled supernatant and add an equal volume of chloroform:isoamyl alcohol. Centrifuge the tube to separate the phases, and transfer the aqueous layer to a fresh tube.

8. Add 0.6 volume isopropanol to the aqueous extract and mix. Allow the mixture to stand at room temperature for 1 hour or overnight.

9. Centrifuge the sample @ 12,000 x g at room temperature and carefully remove the supernatant to discard it. The brown pellet contains DNA and soil humic materials.

10. Allow pellet to dry (either by vacuum or in a laminar flow hood). Rinse the pellet with cold 70% ethanol. Resuspend pellet in 50-100 microliters of TE buffer. This crude extract is ready to be purified.

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[3] A sample of 0.1 gram soil can also be extracted in a 2-ml microcentrifuge tube. Adjust volumes of all reagents accordingly.

II. DNA PURIFICATION

Materials: Electrophoresis chamber and power source; UV light box; razor blades; sterile microcentrifuge tubes; water baths at 70C and 45C; centrifuge; ultracentrifugal filter units (100,000 molecular weight cutoff, Micron Separations, Inc., Westborough, MA).

Reagents: Low-melting SeaPlaque agarose (FMC BioProducts, Rockland, ME); Tris-acetate-EDTA buffer (TAE), pH 8; ethidium bromide staining solution (0.5 micrograms per ml); DNA molecular weight marker; brom-phenol blue loading dye; agarase enzyme (Epicentre Technologies, Inc., Madison, WI).

Procedure:

1. Prepare a 1% SeaPlaque agarose gel with wells large enough to accommodate volumes of crude extracts.

2. Add loading dye to extracts and carefully load into the wells. Run electrophoresis at low to moderate voltage until the brown humic materials have migrated a sufficient distance from the wells.

3. Stain gel in ethidium bromide solution for 10-15 minutes. Destain in Nanopure water for 10 min. Examine gel under low-intensity UV light. High molecular weight DNA will typically migrate only a few millimeters from the wells.

4. Excise the gel bands containing DNA with a razor blade, place in tared tube, and determine gel weight.

5. Follow manufacturer's instructions on use of agarase enzyme to digest gel (e.g., melt gel, add buffer and temper at 45C, add enzyme and digest at 45C for 1-2 hours).

6. Add digested mixture to the top of a filter unit and spin the unit in a centrifuge according to the manufacturer's instructions to wash and concentrate the DNA solution on the filter.

7. Remove the DNA solution from the filter by pipetting and quantitate the DNA by measuring its absorbance at 260 nm. Calculate absorbance ratios at 260/280 nm and 260/230 nm to evaluate contamination by proteins and humic materials, respectively.

Appendix 9-5

PROTOCOL FOR DNA FINGERPRINTING BY VERTICAL TGGE ANALYSIS

1. **Prepare gel mold.** Prepare a mold for the polyacrylamide gel by clamping together 2 glass plates (0.2 cm x 42 cm long x 32 cm wide) separated by 1-mm-thick Teflon spacers placed at the bottom and on the sides. This mold will hold approximately 120 ml of gel.
2. **Make polyacrylamide gel.** Prepare 140 ml of gel solution containing 5% acrylamide, 8 M urea (denaturant), and 0.035% ammonium persulfate in 0.5X Tris-borate-EDTA buffer. Use an acrylamide stock solution containing 40% acrylamide:bisacrylamide (ratio of the mixture is 37.5:1). Immediately prior to pouring the gel into the mold, add 60 microliters of TEMED (N') to catalyze polymerization. Mix the solution carefully to avoid air bubbles. Draw the solution into the barrel of a 50-ml syringe, place the tip of the syringe at the top of the mold between the glass plates, and slowly introduce the solution into the mold. Seal the top of the gel with a comb or another spacer. Allow the gel to cure for at least one hour.
3. **Set up electrophoresis unit.** After the gel has cured in the mold, remove the bottom spacer from the gel. Place the mold upright in the electrophoresis unit with the notched glass plate toward the back facing the upper buffer reservoir. Use silicone sealant between the glass plate and the reservoir to prevent leakage of the buffer. Place plexiglass sheet against the front of the mold and clamp these securely onto the electrophoresis unit. Add 0.5X TBE electrophoresis buffer (45 mM Tris-borate, pH 8, and 1 mM EDTA) to the upper and lower buffer reservoirs of the unit. Make sure that air has been removed from the bottom of the gel mold by using a syringe and bent needle to expel air. Adjust the set point temperature for the heating strip in the lower part of the aluminum block (e.g., 70C) with the digital temperature controller (switches set to vertical gradient). Adjust the set point temperature of the circulating water bath for the cooling channel in the upper part of the block (e.g., 36C). Allow at least 15 minutes for the temperature gradient to be established. Check temperatures of block with thermocouples and recorder.
4. **Load samples onto the gel.** Remove comb from the top of the gel and check buffer level in upper reservoir. Add xylene cyanol dye to the PCR mixtures and load sufficient volumes to obtain 200 ng DNA per well.
5. **Perform electrophoresis under constant voltage.** Connect the unit to the power source to run at 5V per cm gel length (approximately 400 V total). Continue electrophoresis until the xylene cyanol dye is slightly lower than the bottom of the temperature gradient (approximately 10 hours).
6. **Remove gel from mold.** Disconnect the unit from the power source, remove the gel mold from the unit, and place the mold on a horizontal support. Pry off and remove one of the glass plates. Trim gel to desired size.
7. **Develop gel fingerprint**. Stain the gel in either ethidium bromide solution for photography under ultraviolet illumination, or with silver-staining reagents for photography under visible light.

Appendix 9-6

SUMMARY OF PATTERN RECOGNITION ANALYSIS OF FATTY ACID DATA

Philip K. Hopke and Xin-Hua Song
Department of Chemistry, Clarkson University, Potsdam, NY 13699-5810

Data description

There are a total of 245 soil samples analyzed. Each sample is characterized by 26 fatty acids that represent the most abundant phospholipid fatty acids (PLFAs) found in microorganisms. The units of the data are nanomole percent PLFA per gram dry soil. The soil samples are connected with different crops. The identified crop types are listed in Table 1.

Methods

The main goal was to separate different crops related to the soil samples. Two neural network methods, the adaptive resonance theory - based neural network (ART-2a) (Hopke and Song, 1997; Xie *et al.*, 1994) and the Kohonen neural network (Hopke and Song, 1997; Wienke *et al.*, 1994), have been used to treat this data set. Both neural networks can be used to perform an unsupervised pattern recognition examination in which similar soil samples should be grouped together based on the PLFA composition information. In the ART-2a neural network, an adjustable vigilance parameter is used to control the classification resolution. Through the resonance mechanism, the ART-2a can adaptively detect the novel events. In the Kohonen neural network, the high-dimensional data are mapped onto a two-dimensional map through a learning process. Combined with the minimal spanning tree (MST) technique, the Kohonen neural network can provide a visual map representing different classes. In this map, the soil samples with similar PLFA compositions are located in the near neighborhood.

To give the 26 PLFA variables possibly equal weight in the classification, the data were transformed to have zero mean and unit variance (z-transformation) followed by the range scaling which made all values belonging to a given PLFA variable lie between 0 and 1 with respect to the range between the smallest and the largest values.

Results

With an appropriate vigilance parameter (0.95), the ART-2a neural network produced satisfactory classification results that are shown in Table 2. In this case, 34 classes were obtained, of

which 29 classes correspond to the pure crops. These 29 classes of pure crops have been very nicely separated from each other. Although there are 5 mixed classes (a, b, c, d, e) that contain 2 or more types of crops, they are only a minor part of the total samples. Actually, mixed class c can approximately be assigned as the class of crop rotation, because only 1 cotton sample is mixed with the other 11 crop rotation samples. It has been noted that the samples of the same type crop can be classified into more than one pure subgroup. For example, the 112 rice samples were classified into 4 subgroups, with the number of samples in each subgroup 46, 32, 33 and 1, respectively. However, the trained weight matrix of ART-2a can be used to do some diagnostics for the classes, because each weight vector represents the centroid of a class. In this study, the measure of the angle between two weight vectors has been used to indicate the closeness between them. The results show that, in the rice case, the 4 subgroups mentioned above are closest to each other. In the cases of other crops, the similar results have also been obtained.

After the trial and error experience, it was found that 15×15 is a proper size of the Kohonen neural network for mapping the present data set. Figure 1 shows the classification results in the Kohonen map where the number in each neuron position indicates the number of samples activating the corresponding neuron. Figure 2 shows the crop type No. in the Kohonen map where the crop type No. is the same as in Table 1. Here, there are 5 mixed neurons as indicated by the capital letters, A, B, C, D and E. These 5 neurons were activated by the samples of two crop types. Figure 3 shows the minimal spanning tree (MST) connecting all of the active neurons. The MST tree forms the shortest connection graph under the restriction that closed connections are forbidden. Then classification can be made based on the following criterion: the connection between the active neurons with the first largest distance is firstly separated, the connection with the second largest distance is secondly separated, and so on. This process is repeated until a reasonable classification resolution is achieved regarding the separation of different crops. The final classification map with frames representing different classes is shown in Figure 4. There are 15 classes identified by the Kohonen neural network - MST technique. Class a corresponds to tomato. Class b is road. Class c is a mixed group with samples from crop rotation, cotton and pine. Class d is cotton plus fallow, but cotton is dominant. Class e is rice. Class f is fallow. Class g is tomato plus walnut. Class h is crop rotation plus cotton, with the crop rotation dominant. Class i is almond plus fig. Class j is pine. Class k is compost. Class l is almond. Class m is another group of cotton. Class n is construction. Class o is lake. In addition, there are a very minor number of samples spread over the map that either belong to a mixed class or are not classified to any major class. It can be seen that most of the samples have been clearly separated in terms of the crop types.

Conclusions

Two neural networks, the adaptive resonance theory - based neural network (ART-2a) and the Kohonen neural network, have been used to treat the PLFA data from soil samples. The results show that the crop types related to most of the soil samples have been successfully separated from each other.

References

Hopke, P.K. and Song, X.-H., Classification of single particles by neural networks based on the computer-controlled scanning electron microscopy data, *Anal. Chim. Acta*, in press (1997).

Xie, Y., Hopke, P.K. and Wienke, D., Airborne particle classification with a combination of chemical composition and shape index utilizing an adaptive resonance artificial neural network, *Environ. Sci. Technol.*, 28 (1994) 1921-1928.

Wienke, D., Gao, N. and Hopke, P.K., Multiple site receptor modeling with a minimal spanning tree combined with a neural network, *Environ. Sci. Technol.*, 28 (1994) 1023-1030.

Table 1. Identified crop types

Crop type No.	Crop Name	The number of samples
1	rice	112
2	tomato	70
3	fallow	4
4	construction	4
5	pine	2
6	lake	2
7	walnut	3
8	cotton	18
9	almond	11
10	road	2
11	fig	2
12	crop rotation	13
13	compost	2

Table 2. Classification results for the fatty acid data by ART-2a (ρ_max=0.95)

Classified crop	The number of samples in classes
rice	46, 32, 33, 1
tomato	20, 9, 35, 1, 1
fallow	2
construction	1, 1, 1, 1
pine	1, 1
lake	2
walnut	1
cotton	3, 3, 2, 2, 2
almond	5, 3
road	2
fig	---
crop rotation	2
compost	1, 1
mixed class a	4 (cotton) + 1 (fig)
mixed class b	4 (tomato) + 2 (fallow) + 1 (walnut)
mixed class c	11 (crop rotation) + 1 (cotton)
mixed class d	3 (almond) + 1 (fig)
mixed class e	1 (walnut) + 1 (cotton)

Figure captions:

Figure 1. Kohonen map with the number of samples activating the corresponding neurons.

Figure 2. Kohonen map with the crop type Nos. connected with the active neurons. There are 5 mixed neurons as indicated by the capital letters, A, B, C, D and E.
A---Crop type Nos. 5 and 8.
B---Crop type Nos. 2 and 4.
C---Crop type Nos. 2 and 7.
D---Crop type Nos. 7 and 8.
E---Crop type Nos. 4 and 12.

Figure 3. The minimal spanning tree connecting the active neurons.

Figure 4. Classification map with frames representing different classes.

4	0	6	0	2	0	2	1	1	2	0	4	2	2	5
5	2	1	0	1	0	0	0	1	0	0	1	1	2	0
0	2	2	1	2	0	2	0	3	0	0	1	0	2	1
3	1	0	1	1	0	1	0	0	0	3	1	1	1	3
2	1	1	0	2	0	3	0	2	1	0	1	2	1	1
3	1	2	1	2	0	1	0	0	1	1	0	1	0	2
3	1	2	2	1	0	1	0	1	0	1	1	1	2	1
3	1	1	3	0	1	2	0	1	3	1	0	2	1	2
0	1	2	0	0	2	1	0	3	0	1	1	2	0	1
3	3	0	2	1	0	0	1	0	1	3	0	0	3	1
0	1	0	1	0	2	0	2	1	0	1	1	0	0	2
2	0	0	1	0	0	0	1	0	1	2	0	2	0	2
1	1	0	2	0	2	0	2	1	1	1	1	0	0	3
1	1	0	0	0	0	0	0	0	1	0	2	1	0	0
3	2	1	3	0	3	0	2	0	3	1	1	2	0	2

Figure 1

2		2		10		8	8	8	3		1	1	1	1
2	2	2		2				8			1	1	1	
	2	2	12	A		B		8			1		1	1
2	2		8	8		2				1	1	1	1	1
2	2	2		3		C		1	1		1	1	1	1
2	2	2	2	2		7			1	1		1		1
2	2	2	2	2		8		1		1	1	1	1	1
2	2	2	2		12	12		1	1	1		1	1	1
	2	2			12	12		1		1	1	1		1
2	2		12	12			1		1	1			1	1
	11		12		D		1	1		1	1			1
9			5				1		1	1		1		1
11	9		13		8		1	1	1	1	1			1
9	9								1		1	1		
9	9	9	E		8		4		1	1	1	1		6

Figure 2

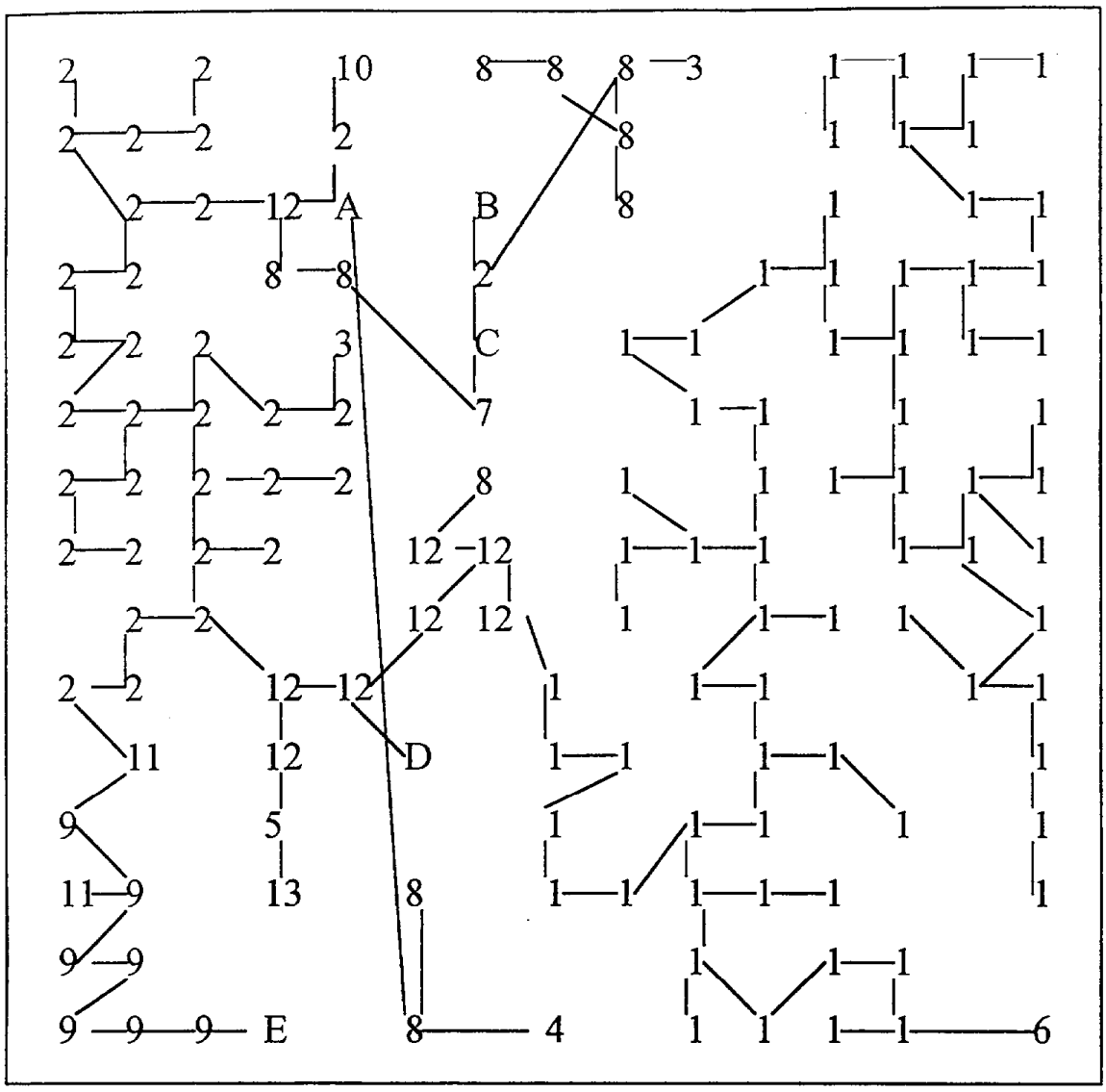
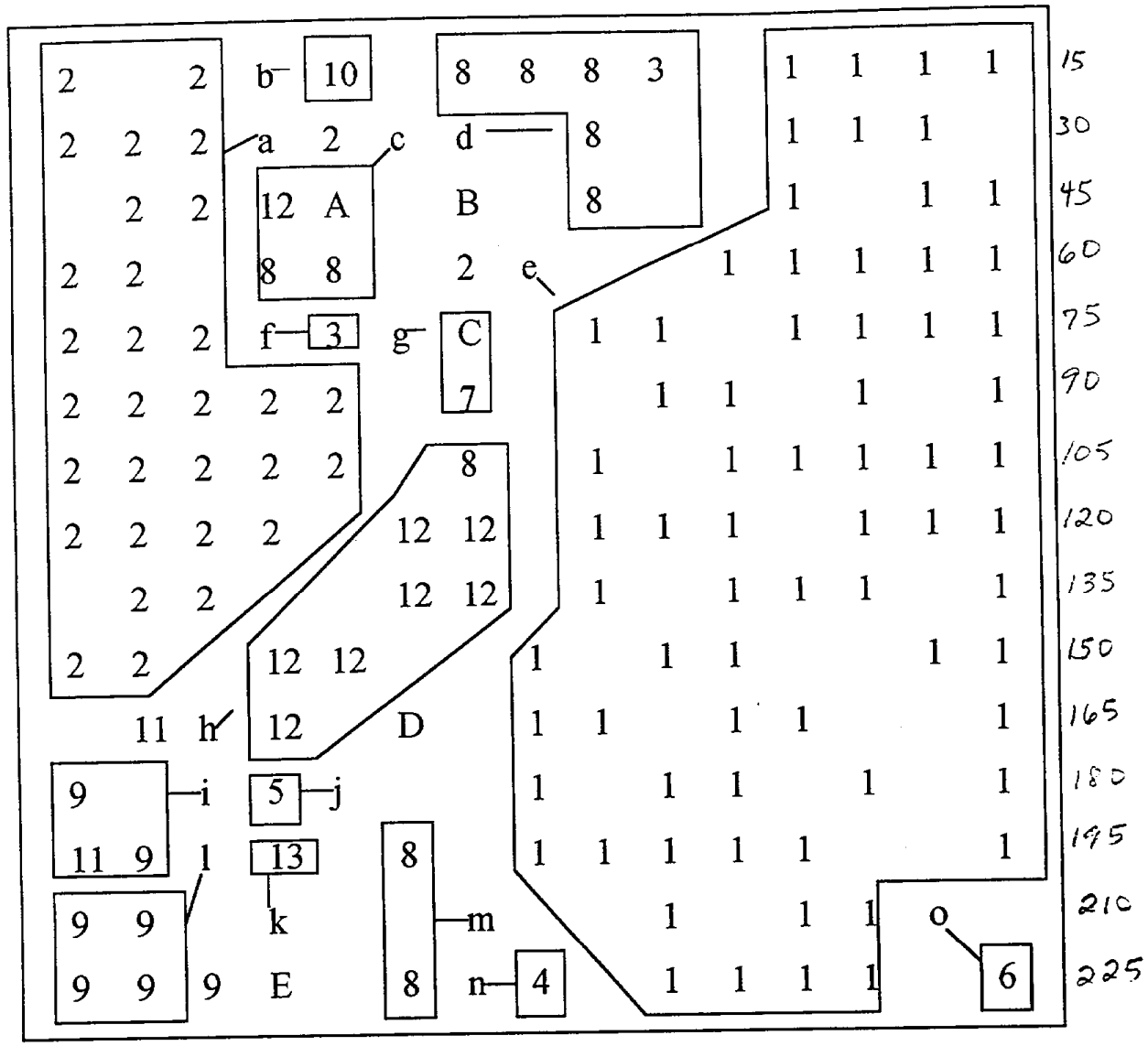

2 2 10 8 8 8 3 1 1 1 1
2 2 2 2 8 1 1 1
2 2 12 A B 8 1 1 1
2 2 8 8 2 1 1 1 1 1
2 2 2 3 C 1 1 1 1 1 1
2 2 2 2 2 7 1 1 1 1
2 2 2 2 2 8 1 1 1 1 1 1
2 2 2 2 12 12 1 1 1 1 1 1
2 2 12 12 1 1 1 1 1
2 2 12 12 1 1 1 1
11 12 D 1 1 1 1 1
9 5 1 1 1 1 1
11 9 13 8 1 1 1 1 1 1
9 9 1 1 1
9 9 9 E 8 4 1 1 1 1 6

Figure 3


2 2 b 10
2 2 2 a 2 c
2 2 12 A
2 2 8 8
2 2 2 f 3 g
2 2 2 2 2
2 2 2 2 2
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2 2 2
2 2
11 h
9 i
11 9 1
9 9
9 9 9
12 12
12 12
12 12
12 D
5 j
13 k
E
8 8 8 3
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Figure 4